

**CARDIOPULMONARY INTERACTIONS OF HYPOXIA AND
HYPERCAPNIA AND THE ROLE OF VASOACTIVE
MEDIATORS IN THE PULMONARY CIRCULATION IN MAN**

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**To Jane, Emma, Laura
and my parents**

This thesis has been composed by David Gerard Kiely and represents the culmination of two years work at the Department of Clinical Pharmacology, Ninewells Hospital, Dundee. The work on which this thesis is based is the candidates own although he was assisted by other members of the department during the conduct of some of the initial studies. This thesis has not been submitted in candidature for any other degree, diploma or qualification.

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ABSTRACT

We have examined the cardiopulmonary effects of hypoxia and hypercapnia in the integrated physiological system of normal man using non-invasive pulsed-wave Doppler echocardiography and phonocardiography and have extended this work to study the role vasoactive mediators in the pulmonary circulation in man.

We have demonstrated that systolic and diastolic function are unaffected by acute hypercapnia in normal man. Acute hypoxaemia significantly impairs both right and left ventricular diastolic function, in a dose dependant manner, although systolic function remains well preserved. In addition to confirming that hypoxia is a potent pulmonary vasoconstrictor we have demonstrated that hypercapnia is a weak pulmonary vasoconstrictor, suggesting a possible role in ventilation perfusion matching in health and disease. We have also shown potentially adverse electrophysiological effects of both hypoxia and hypercapnia, the clinical significance of which is unknown.

The second part of this thesis examines the role of vasoactive mediators in the pulmonary circulation. In a series of placebo controlled studies we have demonstrated for the first time in normal man that angiotensin II is capable of modulating the acute hypoxic pulmonary vasoconstrictor response, using infusions of the non selective angiotensin II receptor blocker saralasin and the orally active type 1 angiotensin II receptor blocker losartan. We have extended this work to patients with hypoxaemic cor pulmonale secondary to chronic obstructive pulmonary disease (COPD) and have

shown beneficial haemodynamic and endocrine sequelae of type 1 angiotensin II receptor blockade in these patients with a vasoreactive pulmonary circulation. These results suggest that manipulation of the renin-angiotensin system may be of therapeutic benefit in this patient group.

We have shown that acute hypoxaemia is a stimulus to endothelin-1 release in normal man and that levels of this peptide are elevated in patients with hypoxaemic cor pulmonale due to COPD. We have demonstrated in a double blind placebo controlled crossover study that systemic infusions of this peptide are capable of causing pulmonary vasoconstriction in a dose dependant manner in addition to having adverse effects on both inotropy and lusitropy in normal man. These novel findings may be important in considering the pathophysiological role of endothelin-1 in both cardiovascular and cardiopulmonary disease. Using an infusion of the nitric oxide inhibitor N^G-monomethyl-L-arginine we have shown for the first time in a double blind placebo controlled crossover study that basal nitric oxide generation is important in maintaining basal pulmonary vascular tone in normal man.

Finally in a study encompassing 114 events of suspected pulmonary thromboembolism (PTE) we have reported for the first time elevated levels of atrial, B-type and N-terminal atrial natriuretic peptides in patients with pulmonary thromboembolism, presumably reflecting the cardiopulmonary sequelae. In addition we have shown significantly elevated levels of these peptides in patients dying of PTE compared to survivors. The results of this pilot study suggest that further work is required to more fully evaluate the diagnostic role of these peptides in PTE and whether they may be

useful in risk stratification and the identification of patients most likely to benefit from thrombolysis.

Our findings from this series of studies are consistent with an important role for vasoactive mediators in modulating pulmonary vascular tone in man in health and disease.

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ABBREVIATIONS

Acc_{mean}	aortic peak acceleration
Acc_{peak}	aortic peak acceleration
ALDO	aldosterone
ANG II	angiotensin II
ANOVA	analysis of variance
ANP	atrial natriuretic peptide
Av_{max}	aortic peak velocity
Av_{max}	maximal velocity of atrial transmitral flow
BNP	b-type natriuretic peptide
CO	cardiac output
CO₂	carbon dioxide
COPD	chronic obstructive pulmonary disease
CSA	cross sectional area
DBP	diastolic arterial blood pressure
ECG	electrocardiogram
EDT	early transmitral flow deceleration time
EDT_c	early transmitral flow deceleration time adjusted for heart rate
ET	left ventricular ejection time
ET CO₂	end tidal carbon dioxide
ET-1	endothelin-1
ET_c	left ventricular ejection time corrected for heart rate
Ev_{max}	maximal velocity of early transmitral flow

HR	heart rate
IQR	interquartile range
IVRT	isovolumic relaxation time
IVRTc	isovolumic relaxation time corrected for heart rate
LVET	left ventricular ejection time
LVETc	left ventricular ejection time corrected for heart rate
MAP	mean arterial blood pressure
MPAP	mean pulmonary artery pressure
N-ANP	N-terminal atrial natriuretic peptide
PAT	pulmonary acceleration time
PCWP	pulmonary capillary wedge pressure
PEP	pre-ejection period
PEPc	pre-ejection period corrected for heart rate
PRA	plasma renin activity
PVR	pulmonary vascular resistance
QS₂	electromechanical systole
QS₂c	electromechanical systole corrected for heart rate
QS₂I	electromechanical systole corrected for heart rate
RAAS	renin-angiotensin-aldosterone system
RAS	renin-angiotensin-system
RIA	radioimmunoassay
SaO₂	oxygen saturation
SBP	systolic arterial blood pressure
SD	standard deviation

SEM	standard error of the mean
SV	stroke volume
SVI	aortic systolic velocity integral
SVR	systemic vascular resistance

**PRESENTATIONS TO LEARNED SOCIETIES AND PUBLICATIONS
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CHAPTER 1

INTRODUCTION

INTRODUCTION

1.1 INTRODUCTION

The pulmonary vascular bed has often been regarded as a passive conduit for the transport of blood from the right side of the heart to the left. Although under normal conditions vasomotor control is minimal and gaseous exchange occurs as a relatively passive process this can be disrupted by a variety of different disease processes. In recent years much interest has been directed towards different cellular elements of the pulmonary vascular tree and the role of endothelial cells in the synthesis and metabolism of vasoactive substances, growth factors and in the regulation of cell growth.

In terms of morbidity and mortality chronic bronchitis and emphysema and pulmonary thromboembolism represent the commonest causes of pulmonary hypertension seen in the general medical setting. The aetiology and pathogenesis of pulmonary hypertension in these conditions is different and although incompletely understood is likely to reflect abnormalities in gaseous exchange, ablation of the pulmonary vascular bed and changes in endothelial function. The discovery of new vasoactive peptides and the characterisation of receptor ligand interactions has given us insight into a variety of different pathophysiological processes and provided us with therapeutic options and diagnostic tests in conditions such as congestive cardiac failure. The role and importance of these peptides in the pulmonary vascular bed is incompletely understood.

This MD Thesis firstly examines the cardiopulmonary effects of hypoxia and hypercapnia in the integrated physiological system of man. Although these abnormalities of gaseous exchange have been extensively investigated in the past the advent of new non invasive methodology has allowed us to more completely elucidate their effects in normal man.

Secondly we have examined the role of vasoactive peptides in the pulmonary circulation. In this respect the interaction of hypoxia with the renin angiotensin system has been of considerable interest and we have examined for the first time the role of angiotensin II in modulating the acute hypoxic pulmonary vasoconstrictor response in normal man and the haemodynamic and endocrine effects of selective angiotensin II receptor blockade in patients with hypoxaemic cor pulmonale. The discovery of endothelin-1 as the most potent vasoconstrictor known to man has heralded much research into its role in conditions characterised by abnormal vasoconstriction. We have examined the interaction between hypoxia and endothelin-1 release and in addition have studied the effects of this hormone on both the pulmonary and cardiovascular systems in normal man. In addition to examining the role of different vasoconstrictors in the pulmonary circulation we have examined whether basal generation of a potent vasodilator, nitric oxide, is essential for maintenance of low pulmonary vascular tone, where conflicting results exist in the current literature.

The final chapter in this thesis examines some of the endocrine sequelae of pulmonary thromboembolism and their possible physiological and diagnostic roles in this condition.

The remainder of this chapter reviews our current understanding of the cardiopulmonary effects of hypoxia and hypercapnia and the role of vasoactive peptides in the pulmonary circulation in man with particular reference to their possible therapeutic and diagnostic value.

1.2 CARDIOPULMONARY EFFECTS OF HYPOXAEMIA

1.2.1 Hypoxic pulmonary vasoconstriction

Hypoxaemia usually arises in man as a consequence of adverse environmental conditions (eg altitude) or due to acute or chronic pulmonary diseases. Since the first description of the phenomenon of acute hypoxic pulmonary vasoconstriction by Von Euler and Liljestrand in 1946 (von Euler US et al, 1946) the effects of hypoxia on the pulmonary circulation have been extensively studied. The mechanism of this response is incompletely understood although teleologically it has beneficial effects regulating foetal blood flow and after birth acting as a homeostatic mechanism to divert blood from areas of alveolar hypoxia and thus maintaining ventilation perfusion homogeneity. The importance of ventilation perfusion matching has been demonstrated in healthy volunteers by Hales (Hales CA et al, 1978) who showed a significant reduction in arterial pO_2 after the intake of a small dose of the vasodilator nitroglycerin. The central regulatory role of this response is reflected by its presence in most animal species (Satchell G, 1962, Millard R et al, 1974, Cueva S et al, 1974). Although the acute effects of this response are undoubtedly beneficial the presence of chronic hypoxaemia can result in elevation of pulmonary arterial pressure, vascular remodelling and over time the development of cor pulmonale. A consequence not uncommonly seen in the context of chronic bronchitis and emphysema.

Pulmonary vasoconstriction is initiated within seconds of the onset of alveolar hypoxia (Jensen K et al, 1992) and is known to be a more effective stimulus to the small pulmonary arteries (the main site of hypoxic pulmonary vasoconstriction) than the

oxygen content of the pulmonary artery perfusate (Marshall C et al, 1983, Bergofsky E et al, 1968). Considerable individual and ethnic differences are seen in the response of the pulmonary vascular tree to hypoxia with the relationship of the curve of pulmonary artery pressure to hypoxaemia being less steep in Himalayan people compared to Andean counterparts who in turn appear to be better adapted to the effects of hypobaric oxygen than their North American counterparts (Williams D, 1994). This is thought to be a reflection of genetic adaptation to hypobaric hypoxia. The inability of some individuals to adapt to hypobaric hypoxia results in a wide variety of disturbances in functions of most organs of the body resulting in the development of "mountain sickness" (Anand IS, 1994).

Nagasaka (Nagasaka Y et al, 1984) showed that hypoxia increased pulmonary vascular resistance in arterial segments upstream from arterioles. Further work with laser technology confirmed hypoxic pulmonary vasoconstriction to small arterioles 30-200µm in diameter (Koyama T et al, 1983). Although the major site of pulmonary vasoconstriction is known the mechanism of this response and how vascular smooth muscles sense hypoxia is poorly understood. Alveolar hypoxia results in calcium influx through voltage dependent calcium channels as a consequence of depolarisation of the resting membrane potential of the smooth muscle cells (Madden J et al, 1985). A number of different vasoactive substances such as angiotensin II (ANG II) (Berkov S, 1974, McMurty I et al, 1984), histamine (Tucker A et al, 1976), serotonin (Miller M et al, 1979), prostaglandins (Weir E et al, 1974) and leukotrienes (McDonnell T et al, 1990) have all been proposed as possible mediators although current interest surrounds the hypothesis that the smooth muscle cells may directly sense changes in

oxygen tension via potassium channels (Post JM et al, 1992). They demonstrated that hypoxaemia inhibited outward potassium current in pulmonary arterial smooth muscles resulting in depolarisation of the membrane potential providing a stimulus for calcium influx through voltage dependent calcium channels and consequently resulting in vasoconstriction.

Although different vasoactive peptides have been excluded as sole mediators of hypoxic pulmonary vasoconstriction (HPV) the interaction between hypoxia and hormonal systems has been of much interest. In this respect the interaction between hypoxaemia and the renin-angiotensin system (RAS) is of considerable interest (Cargill RI et al, 1994). Not only has ANG II been shown to be a potent pulmonary vasoconstrictor (Segel N et al, 1960) but aldosterone may also be important in determining changes in sodium and water homeostasis. This may be particularly relevant where activation of the RAS accompanies chronic hypoxic lung disease, such as occurs in cor pulmonale (Farber MO et al, 1977, Farber MO et al, 1982), where manipulation of the RAS may have therapeutic benefit.

1.2.2 Cardiovascular effects of hypoxia

In addition to effects on the pulmonary vasculature hypoxia affects a variety of other physiological systems. Although the effects of myocardial ischaemia have been extensively investigated much less is known of the effects of arterial hypoxaemia where the majority of studies have examined the effects of hypoxia on isolated heart and muscle preparations. Although acute hypoxaemia has been reported as increasing (Doyle JT et al, 1952, Vogel JA et al, 1967), decreasing (Motley HL et al, 1947) or

having no effect (Fletcher EC et al, 1983) on cardiac output in humans it is now generally accepted that cardiac output rises in a dose dependent fashion with increasing hypoxaemia in healthy men (Phillips BA et al, 1988) and in contrast to its effects on the pulmonary vasculature hypoxia is a weak systemic vasodilator. The time course of cardiovascular response to hypoxaemia is brisk with one study reporting 95% of subjects attaining 85% of maximum cardiac output within 5 minutes (Phillips BA et al, 1988). In this study the increase in cardiac output resulted entirely from an increase in heart rate with no significant changes seen in stroke volume. Indeed this increase in cardiac output is a physiologically predictable response. During hypoxaemia oxygen delivery to the tissues decreases and since oxygen supply to the tissues is a function of arterial oxygenation, cardiac output and haemoglobin, increased cardiac output at least in the acute situation is a teleologically appropriate response. Interestingly ascent to altitude results in an acute increase in cardiac output and heart rate (Vogel JA et al, 1967), however, over a period of 1-10 days heart rate returns to levels slightly above pre-ascent levels, cardiac output and stroke volume decrease to reach a steady state with values 20% below resting levels at sea level (Alexander JK et al, 1967). It is not clear whether this reduction represents a physiological adaptation to improved oxygen delivery to the tissues caused by an increased haematocrit and changes in the oxygen-haemoglobin dissociation curve or reflects impairment of myocardial function. Echocardiography shows a reduction in left ventricular end diastolic and end-systolic diameters although ejection fraction remains normal suggesting a physiological adaptation rather than impaired myocardial function (Alexander JK et al, 1983).

Observational studies in patients have shown that in chronic hypoxic lung disease that left ventricular function at rest is usually normal or increased as judged by left ventricular ejection fraction, cardiac output and pulmonary capillary wedge pressure (Fishman AP et al, 1971, Christianson LC et al, 1979, Anand IS et al, 1992). Interestingly, however, patients with hypoxaemic cor pulmonale have a proportionately smaller increase in cardiac output in response to exercise than do their counterparts with no evidence of pulmonary hypertension (Khaja F et al, 1971). In addition post mortem studies have shown patchy fibrosis and left ventricular hypertrophy in patients with hypoxaemic cor pulmonale (Michelson N, 1980, Kohama A et al, 1990). The functional consequences and of these changes are unknown although one would imagine that they would reduce ventricular compliance and result in impairment of left ventricular diastolic function, changes which have been demonstrated in vivo (Marangoni S et al, 1992).

Studies in healthy humans have demonstrated a significant increase in coronary artery blood flow in response to hypoxaemia with a reduction in inspired oxygen to 10% resulting in a 2 fold increase in coronary blood flow, possibly mediated by prostaglandins (Hellems HK et al, 1957). Ascent to high altitude although resulting in an acute increase in coronary blood flow results in a 25% reduction over the following 7-10 days, presumably reflecting adaptation to improved oxygen delivery (Groover RF et al, 1976). During myocardial ischaemia myocardial performance is dependent on oxygen delivery as well as the build up of metabolites. Left ventricular performance is impaired most by a combination of hypoxaemia and the build up of metabolites as demonstrated by occluding an artery with a percutaneous transluminal

coronary angioplasty balloon. Whereas left ventricular performance is less affected by pacing induced ischaemia (Bruyne B et al, 1993). In patients with chronic lung disease and underlying coronary artery disease the addition of hypoxia to underlying myocardial ischaemia may be enough to precipitate left ventricular failure particularly in the presence of hypercapnia and acidosis. It is not surprising therefore that in addition arrhythmias are common in chronic hypoxic lung disease (Terlapur VG et al, 1982, Holford Fo et al, 1973, Kleiger RE et al, 1974) possibly reflecting direct effects of hypoxia and hypercapnia on the heart as well as myocardial ischaemia. The electrophysiological effects of hypoxaemia in normal humans are not well documented although it is well recognised that there is a close correlation between the level of hypoxaemia and the occurrence of ventricular extrasystoles (Shepard JW Jr et al, 1985). Certainly uncomplicated ascent to altitude and short exposure to hypoxia does not appear to be associated with significant arrhythmias (Vogel JA et al, 1967).

1.3 CARDIOPULMONARY EFFECTS OF HYPERCAPNIA

Hypercapnia occurs in a variety of different disease states commonly occurring in the context of chronic bronchitis and emphysema and also in diseases involving neurological and musculoskeletal systems. It reflects inadequate alveolar ventilation and consequently is usually accompanied by hypoxaemia. However, hypercapnia also occurs in anaesthetic practice in the absence of hypoxia as so called “permissive hypercapnia”. This reflects recent work suggesting that mechanical ventilation may

contribute to barotrauma (Dreyfuss D et al, 1985) resulting in a volume and pressure limited ventilation strategy and higher levels of carbon dioxide (CO₂) as a consequence (Hickling KG et al, 1990, Pesenti A, 1990). In addition to profound effects on the cardiovascular system hypercapnia also affects the respiratory system, central nervous system as well as renal and acid-base physiology. Although hypercapnia may have deleterious consequences patients with respiratory insufficiency can excrete metabolically produced carbon dioxide with a relatively low alveolar ventilation, diminishing ventilatory requirements and thus reducing the work of breathing.

1.3.1 “Direct” and “indirect” effects of hypercapnia

Carbon dioxide can be regarded as having both “direct” “ indirect” circulatory effects in man. Although CO₂ causes dilation of peripheral arterioles and depresses myocardial contractility (Butcher RL et al, 1972, Nejad NS et al, 1967, Price HL, 1960) it stimulates the central nervous system at different levels evoking a variety of sympathoadrenal responses (Cross BA et al, 1962). Firstly we will consider the direct effects of CO₂ on end organs. In isolated guinea pig (McElroy WT Jr et al, 1958), rabbit (Williams EM et al, 1958) and dog heart preparations (Price HL et al, 1955) increased CO₂ concentration results in a reduced rate and force of contraction. The ability of CO₂ to reduce pH of the perfusate ie the ability of CO₂ to form carbonic acid was believed to be primarily responsible for the observed effects, since in the absence of external pH changes force and rate of contraction was independent of CO₂ concentration. A similar effect has been in peripheral blood vessels (Diji A, 1959) although interestingly, pulmonary vessels seem to be the exception to the rule that

acidosis causes vasodilatation (Duke HN, 1949, Bergofsky EH et al, 1962). It has been observed that CO₂ causes vasoconstriction in isolated lungs and this may act as a protective mechanism to direct blood from poorly ventilated areas of the lung in much the same way as hypoxia. The ability of carbon dioxide to dilate capillaries and veins more than arteries and arterioles (Fleishman M et al, 1957) allows control of venous return to the heart and some control over cardiac output. In addition to direct effects, the cardiovascular effects of CO₂ depend on indirect actions mediated via the autonomic nervous system.

It is well known that chemoreceptors in the carotid and aortic bodies are exquisitely sensitive to changes in CO₂ concentration resulting in stimulation of a variety of subcortical areas which themselves can be stimulated directly by CO₂ (Gellhorn E, 1953). The end result is one of cortical stimulation, increased sympathetic discharge and hyperventilation (Price HL, 1960). The indirect effects of CO₂ can therefore antagonise the direct effects with the increased sympathetic discharge mediated centrally having positively chronotropic and inotropic effects in addition to causing arteriolar vasoconstriction. Although breathing low concentrations of inhaled CO₂ 5% had no significant effects on cardiac output in normal man (Burnum JF et al, 1954) an increase was observed with higher concentrations of carbon dioxide (Kilburn KH et al, 1969). This effect is thought to be due to the central nervous system actions of CO₂ mediated via the sympathetic nervous system since blockade of this system with subarachnoid procaine resulted in hypotension and cardiac failure in response to CO₂ inhalation (Lurie AA et al, 1958). Although hypercapnia invariably causes arterial hypertension in the integrated physiological system of man the effects are less

marked than those on cardiac output resulting in a reduction of systemic vascular resistance. This suggests predominance of locally mediated vasodilator over centrally mediated vasoconstrictor effects.

1.3.2 Hypercapnia and the pulmonary circulation

There is some debate concerning the effect of CO₂ on the pulmonary vasculature. The early work of Fishman and colleagues (Fishman AP et al, 1960) examined the effect of inhalation of 3-5% CO₂ on both normal volunteers and patients with chronic bronchitis and emphysema and concluded that in contrast to hypoxia that hypercapnia had no significant effects on the pulmonary vasculature. This work contrasted sharply with animal studies (Duke HN et al, 1949, Bergofsky EH et al, 1962) and the dichotomy was explained by Kilburn and his colleagues (Kilburn KH et al, 1969) who demonstrated pulmonary vasoconstriction in patients with more severe hypercapnia. These findings have been corroborated in other patient studies providing strong evidence for a pulmonary vasoconstrictor effect of hypercapnia (Rosketh R, 1966, Paul G et al, 1964). Breathing 5% CO₂ would appear to be an inadequate stimulus to conscious human subjects due to negation of the effects of carbon dioxide as a consequence of augmented ventilation. One could postulate, however, that inhaling CO₂ in concentrations sufficient to increase arterial CO₂ concentration may cause pulmonary vasoconstriction and be another mechanism in healthy man for maintenance of ventilation perfusion homogeneity.

1.3.3 Arrhythmogenesis

Hypercapnia is known to be arrhythmogenic although in conscious subjects it is well tolerated until CO₂ concentrations exceed twice the normal concentration (Secher PH et al, 1960). It is well known that anaesthetics can change the threshold at which arrhythmias occur in response to CO₂ (Price HL, 1958). Interestingly anaesthetics that increase the sympatheticoadrenal response lower the arrhythmia threshold to CO₂ (Lurie AA et al, 1958). This finding in conjunction with the observation that arrhythmias observed during CO₂ inhalation can be reproduced by direct stimulation of cardiac nerves or the addition of adrenaline, suggests that it is the sympathoadrenal stimulation caused by CO₂ that is responsible for its arrhythmogenic potential (Price HL, 1958). Although asystole has been observed in the context of hypercapnia it is known that hypercapnia is itself not responsible for this phenomenon but that it exaggerates the cardiac response to vagal stimulation (Clowes G Jr et al, 1955). Although arrhythmias are common in patients with hypercapnia who have underlying chronic bronchitis and emphysema the importance of hypercapnia per se and the possible synergism between hypoxia and hypercapnia has not been fully investigated.

1.4 THE ENDOTHELIUM AND MODULATION OF PULMONARY VASCULAR TONE DURING NORMOXAEMIA AND HYPOXAEMIA.

The vascular endothelium acts not only as a barrier between vascular smooth muscle and blood but has a variety of well defined functions. These include processing of circulating hormones, preservation of a non-thrombotic surface and regulation of pulmonary vascular tone and smooth muscle proliferation (Vane JR et al, 1990, Dzau VJ et al, 1991, Ogawa S et al, 1990, Scott-Burden T et al, 1993). Evidence suggests that endothelial dysfunction plays a central role in contributing to the development of pulmonary hypertension due to an imbalance of vasodilators/vasoconstrictors (Christman BW et al, 1992) and growth inhibiting/promoting factors (Dzau VJ et al, 1991). A variety of different stimuli such as hypoxia and shear stress are known to cause endothelial dysfunction and pulmonary hypertension is likely to represent a combination of both endothelial initiated and mediated responses. It is currently thought that basal generation of vasodilators maintains the low pressure pulmonary circulation and protects it from the development of pulmonary hypertension and that under normal conditions this vascular bed is well protected from vasoconstrictor substances. Indeed the observation that in the development of hypoxic pulmonary hypertension levels of endothelial derived constricting factors such as ET-1 (Giadi et al, 1993) and ANG II are increased whilst the production and or effects of vasodilators such as nitric oxide (NO) (Dinh-Xuan AT et al, 1991, Adnot S et al, 1991) and prostacyclin (Christman BW et al, 1992) are impaired are consistent with this hypothesis.

1.4.1 Vasodilators

Prostacyclin was the first identified vasodilator synthesised by endothelial cells and its release has been shown to be stimulated by hypoxaemia. Interestingly inhibition of prostaglandin synthesis has been shown to enhance the acute HPV response to alveolar hypoxia in an animal model (Weir EK et al, 1978) and increase pulmonary vascular resistance in patients with chronic bronchitis and emphysema (Adnot S et al, 1987). This suggests that prostaglandins may play a role in modulating HPV.

Furchgott and Zawadski (Furchgott RF et al, 1980) isolated a second endothelial derived relaxing factor in 1980 which has subsequently been identified as NO. Although it is well established that impairment of endogenous NO release whether induced pharmacologically or due to structural endothelial disease is associated with an increased response to vasoconstrictor substances (Adnot S et al, 1991, Dinh-Xuan AT et al, 1991) there is still some debate regarding whether basal generation of NO is essential for maintenance of low pulmonary vascular tone where conflicting results exist suggesting the possibility of species specificity. In the cat (McMahon TJ et al, 1991) and lamb (Fineman JR et al, 1991, Fineman JR et al, 1992) inhibition of NO synthesis is associated with an increase in pulmonary vascular tone whereas studies in the rat (Hasunuma K et al, 1999), dog (Archer SL et al, 1990, Nishiwaki K et al, 1992) and rabbit (Cherry PD et al, 1987) have shown either no or variable effect. To date only one study in normal man has been performed suggesting an important role for basal NO synthesis in maintenance of both systemic and pulmonary vascular tone with

an infusion of N^G-monomethyl-L-arginine significantly increasing both systemic and pulmonary vascular resistance (Stammeler JS et al, 1994).

Nitric oxide also plays an important role in modulating HPV. Animal studies using isolated lungs from rats have shown that inhibitors of NO such as methylene blue and L-arginine potentiate hypoxic pulmonary vasoconstriction (Brashers VL et al, 1988) and endothelium derived relaxing factor inhibits HPV in rats (Liu S et al, 1993). Studies in normal man have shown that inhaled NO selectively reverses HPV (Frostell CG et al, 1993) and this coupled with the well documented increase in NO production in response to hypoxaemia (Busse R et al, 1993) suggests an important role for NO in modulating the acute HPV response. Impairment of endothelium dependent pulmonary artery relaxation has been demonstrated in arteries from patients with end stage chronic obstructive pulmonary disease (COPD) (Dinh-Xuan AT et al, 1991). Compared to control subjects removal of endothelial production of NO eliminates the difference in tension in the arterial rings increasing the tension in control rings but not in patients, suggesting that NO release which normally acts as a brake on vasoconstriction is reduced in arterial rings from patients with chronic obstructive airways disease favouring not only impaired relaxation but also favouring excessive constriction of pulmonary arteries in these patients. Pepke-Zaba has demonstrated that inhaled NO can be used as a selective pulmonary vasodilator in patients with moderately severe pulmonary hypertension (Pepke-Zaba et al, 1991) and studies are currently underway examining whether inhaled NO may have a role to play in the treatment of patients with end stage COPD. In addition a complex interplay also exists between NO and ET-1 and NO may play an important role in modulating pulmonary

vascular tone by reducing ET-1 synthesis and release (Boulanger C et al, 1990). This may be of particular importance in disease states characterised by excessive vasoconstriction and elevated levels of this peptide.

In addition to locally generated vasodilators a number of other substances produced at distant sites are known to have important effects on the pulmonary vascular bed. Of particular interest is a family of peptides known as the natriuretic peptides which cause natriuresis, vasodilatation and suppression of the renin angiotensin system (Struthers AD et al, 1994). ANP or atrial natriuretic peptide and BNP or B-type natriuretic peptide are synthesised from primarily atrial and ventricular myocytes respectively and are secreted in response to changes in wall tension (Yasue H et al, 1994) and hypoxaemia and may play an important role in autoregulation particularly in the context of pulmonary hypertension. Levels of ANP have been shown to be elevated during acute hypoxaemia in normal man (Lawrence DL et al, 1990), in primary pulmonary hypertension (Morice AH et al 1990) and hypoxaemic cor pulmonale (Lang CC et al, 1992).

In terms of modulating the pulmonary vascular response to hypoxaemia ANP has been shown to cause potent inhibition of HPV in isolated perfused rat lungs (Ou LC et al, 1989, Zhao L et al, 1992) and ANP has been shown to prevent the pulmonary vascular remodelling seen in response to chronic hypoxia in rats (Zhao L et al, 1991). This response again appears to be species related with no effect seen on HPV in dogs. Studies in have shown that both ANP and BNP cause pulmonary vasodilatation in normal man in response to pressor agents (Cargill RI et al, 1996) and BNP but not

ANP was able to attenuate the acute HPV response in normal man (Cargill RI et al, 1995). In patients with COPD infusions of ANP have been shown to cause vasodilatation (RogersTK et al, 1994, Adnot S et al, 1989). In the same way as NO production may ameliorate the effects of other vasoconstrictors it has been proposed that the vasorelaxant and natriuretic properties of the natriuretic peptide system may antagonise the effects of RAS activation which is commonly seen in the context of hypoxaemic cor pulmonale secondary to COPD.

1.4.2 Vasoconstrictors

Following the identification of endothelial derived relaxing factors investigators turned their attention to searching for endothelial derived constricting factors. This resulted in the identification of a 21 amino acid peptide which was isolated and sequenced by Yanagisawa in 1988 and called endothelin (Yanagisawa M et al, 1988). This peptide causes long acting vasoconstriction and is the most potent pressor agent known to man. Further work identified two other structurally related peptides. ET-1 and endothelin-2 (ET-2) exhibit the closest structural similarity and are more potent vasoconstrictors than endothelin-3 (ET-3) (Inoue A et al, 1989). In the lung both ET-1 and ET-3 are abundantly expressed (Giad A et al, 1991, Filep JG, 1993) and both have been shown to constrict pulmonary arteries and veins from various animal species (Filep JG, 1993) and ET-1 has been shown to have mitogenic properties on vascular smooth muscle cells, consistent with the hypothesis that ET-1 plays a role in vascular remodelling (Janakidevi K et al, 1992). Receptors have been isolated in smooth muscle cells (Hirata Y et al, 1988, Hosoda K et al, 1991) where stimulation of the type A endothelin receptor results in gradual onset but long lasting constriction

whilst stimulation of the type B receptor causes transient vasodilatation although it is also involved in the vasoconstrictor response (Bigaud M et al, 1992, Clozel M et al, 1992). Synthesis of ET-1 on the basis of induction of mRNA or increased levels of ET-1 is stimulated by a variety of physical and chemical stimuli in vitro including exposure to adrenaline, thrombin, vasoactive peptides such as ANG II and by physical stimuli including hypoxia and shear stress (Haynes WG et al, 1993). In rats hypoxia stimulates ET-1 mRNA expression (Kourembanas S et al, Elton TS et al, 1992) and release of ET-1 from perfused resistance vessels (Rakugi H et al, 1990). In vitro ET-1 has been shown to cause pulmonary vasoconstriction in rat and human pulmonary artery resistance vessels (Crawley D et al, 1989, MacLean MR et al, 1994) and ET-1 has been shown to have both vasoconstrictor and vasodilator properties in the rat lung (Hasanuma K et al, 1990). An important role for ET-1 in modulating the acute HPV response in rats has been shown by Oparil and workers (Oparil S et al, 1995) who have shown that endothelin receptor blockers attenuate this response and Eddahibi and colleagues (Eddahibi S et al, 1995) have shown that endothelin receptor blockade can attenuate the development of pulmonary hypertension in chronically hypoxic rats. Interestingly the hypoxia induced contraction of canine pulmonary artery rings was not antagonised by the selective type A endothelin antagonist BQ-123 (Douglas SA et al, 1993) suggesting species specificity.

It is thought that ET-1 is not stored intracellularly reflected by the observation that production of ET-1 by cultured endothelial cells is not detected until at least 30 minutes stimulation with thrombin (Yanagisawa M et al, 1988). It would seem unlikely therefore that ET-1 is a mediator of hypoxic pulmonary vasoconstriction

particularly given the long time course of its actions (Vanhoutte PM et al, 1989). However, evidence does suggest that it is capable of modulating the acute HPV response as a consequence of both hypoxia mediated ET-1 release and hypoxia mediated increases in maximum ET-1 binding (Liu J et al, 1990). These interactions as well as the interactions of ET-1 with other vasoconstrictors (Emoriti CI et al, 1991) and vasodilators (Suzuki S et al, 1991, Warner TD et al, 1989) suggests that ET-1 may play an important role in modulating the vascular response to hypoxaemia particularly in conditions where ET-1 levels are elevated. However, further studies need to be performed in man to evaluate more fully the role of ET-1. In particular although levels of ET-1 are known to be elevated in primary pulmonary hypertension (Stewart DJ et al, 1991), the effects of hypoxaemia on ET-1 release and the effects of ET-1 on the pulmonary vascular bed in normal man are not known. It has still not been established whether levels of ET-1 are elevated in hypoxaemic cor pulmonale where conceivably they could enhance pulmonary vasoconstriction and increase right ventricular afterload.

The octapeptide ANG II is a potent systemic and pulmonary vasoconstrictor (Segel N et al, 1960) and is formed from angiotensin I (ANG I) by the action of angiotensin converting enzyme (ACE). This enzyme is predominantly a tissue enzyme and is localised mainly to the surface of endothelial cells in the systemic and pulmonary circulations. Highest concentrations of this enzyme are found in the lung (Ryan JW et al, 1975) on the luminal surface of the pulmonary vascular endothelium. Just under half of arterial ANG II is formed by the pulmonary circulation with approximately 60-

80% of circulating ANG I being converted to ANG II by a single pass through the lungs (Ng KKF et al, 1967, Ng KKF et al, 1968). During hypoxaemia, blood flow through the lungs is accelerated and the conversion of ANG I becomes less efficient (Szidon et al, 1980). ANG II receptors have been demonstrated in the pulmonary circulation. Although a number of ANG II receptors have been identified most of the vascular effects are thought to be modulated via the AT₁ receptor (Timmermans PBMWM et al, 1991). The close association of ACE with the endothelial cells of the pulmonary circulation, resulting in the conversion of ANG I to its active metabolite, is ideally suited to regulate blood flow to the lung. If the rate of conversion of ANG I to ANG II can be controlled then the blood flow to that area could be regulated by the amount of ANG II produced. Although it has been demonstrated that ACE activity in the lung can be enhanced after short term exposure of rat pulmonary artery to hypoxaemia (Krulowitz AH et al, 1984) these results are in sharp contrast to longer term studies showing a reduction in ACE activity at 14 and 28 days (Jin H et al, 1987). Interestingly although there is a reduced pressor response to ANG I, there is restoration of the response to infused ANG II reflecting receptor up regulation in the face of reduced enzymatic activity.

ANG II in addition to direct pulmonary vasoconstrictor effects has been shown to potentiate the HPV response in isolated perfused rat lung. Berkov postulated that ANG II was required to facilitate hypoxic pulmonary vasoconstriction (Berkov S, 1974) although McMurty subsequently showed that other pulmonary pressor agents also possessed the ability to restore HPV in saline perfused rat lung (McMurty IF, 1984). In vivo studies have shown that ANG II potentiated acute hypoxic pulmonary

vasoconstriction in dogs (Alexander JM et al, 1976) although other workers have found that chronic infusion of ANG II paradoxically attenuated pulmonary hypertension and vascular remodelling in chronically hypoxic rats (Rabinovitch M et al, 1988). Interestingly prior exposure to chronic hypoxia appears to sensitise the pulmonary vasculature to the acute pulmonary pressor effects of ANG II in rats (Caldwell RW et al, 1981). Studies in humans are rare although a recent study has shown that although ANG II is a potent pulmonary pressor agent when given systemically to normal man and the pulmonary pressor effect of angiotensin II is blunted in the presence of hypoxaemia (Cargill RI et al, 1994). This may simply represent a geometric phenomenon due to large differences in pulmonary vascular tone during normoxaemia and acute hypoxaemia rather than representing a specific interaction between these two pressor stimuli. It may potentially be important in situations where hypoxaemia and elevated levels of ANG II co-exist such as cor pulmonale. Where alleviating reversibly HPV with oxygen therapy could allow endogenous ANG II to elicit proportionately greater pulmonary vasoconstriction and hence limit the potential benefits of oxygen therapy.

ACE inhibitors and ANG II antagonists have given us some insight into the role of ANG II during hypoxaemia. The competitive ANG II antagonist, saralasin when administered to dogs had no significant effects on HPV (Hales CA et al, 1977) although in conditions where ANG II levels are not elevated saralasin behaves as a partial agonist (Fagard R et al, 1980). Studies performed with ACE inhibitors in the cat (Prewitt RL et al, 1981) have shown that although they were unable to attenuate the acute HPV response they did attenuate the development of pulmonary

hypertension and vascular remodelling in chronically hypoxic rats (Zakheim RM et al, 1975) possibly an effect secondary to inhibition of the well known mitogenic properties of ANG II.

Interestingly patients with hypoxaemic cor pulmonale have activation of the RAS. Although treatments such as diuretics (Burnier M et al, 1992) cause activation of the RAS, even in the absence of diuretic therapy, Mannix et al demonstrated activation of the RAS in cor pulmonale (Mannix ET et al, 1990). The stimulus for RAS activation in this patient group remains unclear although observations suggest that hypercapnia may play a central role. In the absence of hypercapnia, RAS activation in hypoxaemic patients is rare (Farber MO et al, 1982) and indeed hypoxaemia has not been shown to increase circulating ANG II levels in normal man although these may not represent accurately ANG II levels at a tissue level. This would suggest an aetiological role for combined effects of hypercapnia with hypoxia and may relate to the following observations. Firstly, hypercapnia is a potent systemic vasodilator and during exacerbations of COPD this is associated with a reduction in systemic vascular resistance (Anand IS et al, 1992). Thus, although these patients have normal or increased cardiac output (Anand IS et al, 1992, Reihmann DH et al, 1985) they appear to have severely reduced renal blood flow and glomerular filtration rate (Farber MO et al, 1982, Anand IS et al, 1992). It is therefore an attractive hypothesis to suggest that in the presence of increased right ventricular afterload, a consequence of pulmonary hypertension, that the heart is unable to compensate for falling systemic vascular resistance by further increments in cardiac output. This pattern has been observed during exercise where patients with cor pulmonale had a proportionately smaller cardiac output response to exercise compared with COPD patients without pulmonary hypertension (Khaja F et al, 1971). This may lead to further reductions in renal blood flow and direct stimulation of the RAS. ANG II may then sustain this vicious circle by causing further pulmonary vasoconstriction (Segel N et al, 1960),

and perhaps interact adversely with HPV. In terms of the haemodynamic effects of blood gas abnormalities, it may also be relevant that hypercapnia, as well as causing systemic vasodilatation may also induce some degree of pulmonary vasoconstriction (Kilburn KH et al, 1969) whilst hypoxia which causes marked pulmonary vasoconstriction, may also contribute to systemic vasodilatation in patients with COPD (Anand IS et al, 1992).

Hypercapnia and hypoxia have also been studied in relation to direct effects on renal function. Kilburn et al studied the effects of hypoxia and hypercapnia on renal function in patients with COPD recovering from episodes of respiratory failure (Kilburn KH et al, 1971). In this study, decreasing arterial oxygen tension led to an initial increase in renal plasma flow and glomerular filtration rate (GFR), presumably a result of intra-renal autoregulation, but with further decreases in oxygenation, a decline in renal function was observed. Severe hypercapnia also caused renal blood flow and GFR to decrease. It would appear therefore in cor pulmonale that although hypoxaemia and hypercapnia can individually influence the RAS, both may need to be present in synergistic fashion to produce clinically detectable RAS activation and consequent fluid retention.

In view of evidence suggesting that angiotensin II may have detrimental pulmonary pressor effects in hypoxaemic cor pulmonale a number of small acute dosing studies have been performed (Boschetti E et al, 1985, Takade K et al, 1986, Bertoli L et al, 1986, Zielinski J et al, 1986, Patakas D et al, 1988, Peacock AJ et al, 1992, Burke CM et al, 1985, Pison CM et al, 1991). These have been limited by small patient numbers and inconsistent inclusion criteria which may explain their variable effect on pulmonary haemodynamics. The use of vasodilators in this condition is also controversial due to concern over worsening of ventilation perfusion mismatching

although this may be offset by improvements in cardiac output and oxygen delivery to the tissues.

The exciting advent of new more specific ANG II receptor antagonists will allow us to more selectively and specifically block the effects of angiotensin II and help us to more accurately evaluate the role of this peptide in the HPV response in health and disease.

CHAPTER 2

METHODS

METHODS

2.1 INTRODUCTION

The work encompassed in this thesis includes both studies in normal man as well as patient studies. In view of ethical considerations and repeated interventions in our study population our measurements were made using non-invasive methodology. This has given us insight into hitherto unknown cardiovascular effects and neurohormonal interactions in normal man. This methodology, however, has associated limitations which will be alluded to in the text.

This chapter includes a review of different methods available for measuring systemic and pulmonary haemodynamics and cardiac function with particular reference to the methods employed during the conduct of the studies included in this thesis.

2.2 SYSTEMIC HAEMODYNAMICS

2.2.1 Measurement of heart rate and blood pressure

Heart rate, systolic arterial blood pressure, mean arterial blood pressure and diastolic arterial blood pressure were measured using a semiautomatic sphygmomanometer (Vital Signs Monitor, Critikon, Tampa, FL, USA). The mean of three consistent readings was taken at each time point. Where electrocardiographic measurements

were made heart rate was calculated from the electrocardiogram from the mean of at least five R-R intervals.

2.2.2 Cardiac output

Cardiac output (CO) can be assessed using a variety of different direct and indirect methods. The commonest used direct methods include the Fick method, thermodilution and dye dilution techniques. The Fick method is regarded by many as the gold standard. This technique measures the amount of oxygen taken up by the volume of blood passing through the lungs in one minute as a measure of CO. This technique however, requires a number of invasive procedures including right heart catheterisation to measure mixed venous oxygen concentration, arterial puncture to measure the oxygen content of arterial blood and analysis of inspired and expired gases. In addition the Fick equation cannot be accurately applied whilst oxygen uptake is changing and as such calculations using this method may not be valid in this situation (Fishman AP et al, 1952, Zierler KL et al, 1991). Dye dilution also requires right heart catheterisation and insertion of an arterial line but is limited by the number of measurements that can be made due to accumulation of the dye injection. Thermodilution techniques although requiring right heart catheterisation do not require insertion of an arterial line and allow repeated measures to be taken.

In addition to these direct methods a number of non-invasive indirect methods have been used and validated against these invasive measures. These include the use of Doppler echocardiography (Coats AJS, 1990) the indirect Fick method using carbon

dioxide as the indicator (Defares JG, 1968, Clausen JP et al, 1970) and impedance cardiography (Appel PJ et al, 1986, Northridge DB et al, 1990). Impedance cardiography measures the electrical impedance between electrodes located in the neck and thorax. A current is passed between electrodes around the neck and thorax and as blood leaves the thorax during the cardiac cycle the impedance of the system changes allowing calculation of CO. The indirect Fick method uses CO as the indicator and although allowing repeated measurements to be made it makes assumptions regarding steady state conditions regarding gaseous exchange.

We have, however, chosen Doppler echocardiography to calculate CO. It has been shown to be a valid measure when compared with invasive measures, is reproducible, can be applied during states of changing gaseous exchange and is non-invasive, allowing repeated measurements to be made and the response to a variety of different interventions assessed without the associated risks of invasive monitoring.

Mathematical considerations

Volumetric flow rate (Q) may be calculated as $Q(t) = V(t) \times CSA$ where v is the spatial mean blood velocity, CSA is cross sectional area of the vessel and t is time. Therefore to calculate stroke volume (SV) from ascending aortic blood flow $SV = \text{spatial mean aortic blood flow} \times \text{ejection time} \times CSA \text{ of aorta}$. Inherent in this approach are certain assumptions that the total forward flow during systole equals the net stroke volume and that the cross sectional size of the aorta equals the cross sectional area of flow. For the purpose of our studies patients with aortic regurgitation and or stenosis were excluded. The spatial mean aortic velocity can then

be obtained from the mean Doppler shift if the artery is uniformly insonated. The narrow ultrasound beam, however, only insonates part of the cross sectional area of the aorta. For a flat velocity profile the spatial mean velocity will be close to the spatial maximum of the velocity in the centre of the lumen. The maximum velocity measured by the narrow Doppler beam, therefore approximates the spatial mean velocity assuming that the velocity profile in the aorta is fairly flat. Studies in animals have shown that the velocity profile in the aorta is fairly flat (Seed WA et al, 1971) and thus we can assume that the Doppler measured maximum velocity approximates the spatial mean velocity. Allowing for the above assumptions and an angle of less than 25° , the traversed distance of blood may be obtained by integrating the velocity measured over systole.

Doppler technique

The Doppler recordings were made using a Vingmed SD50 (Vingmed Sound, Horten, Norway) in either pulsed or continuous wave mode. When used in pulsed mode the sample volume of the cylinder is approximately 5 mm in depth and 5mm in length. The received signals are processed by frequency estimators and converted into analogue voltages proportional to mean and maximal frequency shifts. These estimators have been shown to determine the mean and maximal velocities accurately in experimental studies and also in the human aorta (Angelsen BAJ et al, 1976, Brubakk AO et al, 1977). The integrator accurately calculates the velocity integral. For the purposes of analysis we integrated the maximal velocity curve as this has been shown to be influenced less by aiming errors than mean velocity (Brubakk AO et al, 1982).

The Doppler probe was placed in the suprasternal notch and the angle was adjusted until flow signals were obtained in the ascending aorta using continuous mode. The pulsed wave was then used and the depth and angle adjusted to achieve maximal velocity and clearly defined flow velocity envelopes. All normal volunteers and patients were screened prior to inclusion in the studies to ensure good echocardiographic windows and Doppler flow profiles. The criteria for acceptance of an adequate signal were the presence of a high frequency whistling sound when listening to the audible signal and a smooth velocity envelope characterised by a rapid increase to peak velocity (Hatle L et al, 1982) . The maximal velocity was integrated automatically through each systole and calculations were based on at least 10 consecutive beats measured whilst an adequate signal was obtained. The aortic systolic velocity integral was obtained as described above and on-line calculations of stroke volume ($SV = SVI \times CSA$) and CO as the product of SV and HR were also made. Cross sectional area of the aorta was measured at the level of the aortic root using M-mode echocardiography.

Validation and reproducibility of Doppler measures of cardiac output.

Several studies have been performed in patients and normal volunteers and show a good correlation between both pulsed wave and continuous wave Doppler measures of CO and those measured invasively during right heart catheterisation using either the Fick principle or thermodilution methods. We have used pulsed wave to measure maximal aortic velocity. We found as have others that a clear envelope is more easily obtained using pulsed wave echocardiography than continuous mode (Ihlen H et al,

1984). In addition we find the pulsed wave technique more preferential since continuous mode sends velocity information from all along the Doppler beam and the velocities measured may not be from the aortic root. These Doppler measures have also been shown to be reproducible in patients and in normal healthy subjects with coefficients of variability of consistently less than 10% (Coats AJS, 1990) which concur with our own published coefficients of variability (Lipworth BJ et al, 1994, Kiely DG et al 1997a, Kiely DG et al, 1997b). The largest source of error in Doppler estimation of CO is generally accepted as measurement of cross sectional aortic area (Ihlen H et al, 1984). Although the accurate determination of diameter is not imperative to study relative changes in stroke volume and CO as was the case in our series of studies it is very important for an absolute measure. In this respect the aortic diameter of our study patients was kept as a constant during placebo and active treatments days. It is known that cross sectional area is a function of pressure and studies in man indicate that it can change $\pm 3\%$ to $\pm 12\%$ during the normal cardiac cycle (Greenfield JC et al, 1962, Merillon JP et al, 1978). We measured the aortic root diameter during early systole using M-mode echocardiography. Studies have shown this measurement to be reproducible and CO calculated from it to have a good correlation with catheter measures (Loeppky JA et al, 1984) and to be significantly better than predictions of diameter based on body surface area (Francis GS et al, 1973). Indeed, Goldberg and workers showed that aortic diameter measurements by echocardiography are not apparently different from those measured during angiography (Goldberg SJ et al, 1982). During serial visits in both our normal volunteer and patient studies the pulsed wave Doppler measurements were made at the same depth and although this is likely to improve the reproducibility of results

studies have shown that the sampling site for blood velocities is of significantly less importance than the level at which the cross sectional area is measured in terms of influencing the measured CO (Fischer DC et al, 1983). Indeed Ihlen and colleagues found that although measurements of cross sectional area differed at different sampling sites the maximal obtained velocities were no different at the aortic orifice, proximal and distal aortic root (Ihlen H et al, 1984). Table 2.1 includes a representative sample of studies comparing Doppler measures of CO with those measured using the Fick method and thermodilution techniques and evaluating the reproducibility of these measures. In addition to a good correlation between Doppler measures and invasive measures of CO these studies have also shown that Doppler echocardiography is capable of detecting changes in CO after vasodilator therapy. Rose et al (Rose JS et al, 1984) studied the effect of a number of different vasodilators on CO and found a good correlation between baseline measures of CO using continuous wave Doppler echocardiography and thermodilution techniques at baseline ($r=0.92$) and also after intervention ($r=0.88$) and a similar percentage change from control to intervention; Doppler $32\pm11\%$ versus thermodilution $28\pm9\%$. In addition except for one patient the directional changes in CO by the two techniques were identical.

As previously alluded to there are a number of potential errors when using the Doppler technique and in particular although vasodilators may theoretically affect the diameter of the aorta Rose et al found little difference between his correlation coefficients found before and after intervention assuming the diameter of the aorta remained unchanged during vasodilator therapy. This suggests that the assumption

that the diameter of the aorta would remain constant during vasodilator therapy is unlikely to introduce a significant error into estimation of CO using Doppler echocardiography. Studies have demonstrated that adequate Doppler signals can be acquired in 85-90 % of unselected patients (Huntsman et al, 1983, Ihlen et al, 1984). This table also includes the published results of our own reproducibility using this technique in both patient and normal volunteer studies.

Table 2.1. Validation and reproducibility of Doppler echocardiographic measures of cardiac output

Loeppky JA et al 1984	Fick method pulsed mode	r=0.84	n=15 m=15		angina pectoris
Ihlen H et al 1984	Thermodilution pulsed mode	r=0.96	n=10 m=20		angina pectoris
	Fick method pulsed mode	r=0.90	n=11 m=11		angina pectoris
Huntsman LL et al 1983	Thermodilution continuous mode	r=0.94,	n=45 m=110	CV=9.4%	ITU patients
Robson SC et al 1988	continuous mode	n/a	n=8 m=32	CV=8.8%	normals
Voyles WF et al 1982		n/a	n=10 m=200	CV=9-10%	normals
Gisvold SE et al 1982		n/a	n=5 m=50	CV=6-11%	normals
Kiely DG et al 1997a	pulsed mode	n/a	n=10 m=60	CV=9.3%	normals
Kiely DG et al 1997b	pulsed mode	n/a	n=8 m=24	CV=9.6%	COPD

A summary of technique employed and patient group studied is given (r=correlation coefficient, n=number of patients studied, m=number of measurements made, CV= coefficient of variation, COPD=chronic obstructive pulmonary disease).

2.2.3 Systemic vascular resistance

For the calculation of systemic vascular resistance an assumption was made that the mean right atrial pressure was zero. Systemic vascular resistance was expressed in dyne.s.cm⁻⁵; systemic vascular resistance = (mean arterial blood pressure/cardiac output) x 80.

2.3 PULMONARY HAEMODYNAMICS

2.3.1 Non invasive measures of pulmonary artery pressure

The reluctance to repeatedly catheterise patients to establish the degree of pulmonary hypertension and to follow its course has resulted in the development of a variety of different techniques to estimate systolic (SPAP) and mean pulmonary artery pressure (MPAP) using non-invasive means. Initially M-mode and 2D-echocardiography were developed but have these have now been superseded by the development of Doppler echocardiography which is the most direct and accurate echocardiographic technique for assessing blood flow. Broadly speaking there are two different Doppler methods for estimating pulmonary artery pressure. Firstly, assessment of trans tricuspid gradient by continuous mode Doppler echocardiography can be used to measure systolic pulmonary artery pressure, is a direct measure and has a good correlation with catheter measures. We have however, used right systolic time intervals in particular pulmonary acceleration time to estimate MPAP in our series of studies. Although this estimation is based on correlation rather than being a direct measure it has a good correlation with catheter measures of MPAP and has the advantage that pulmonary

flow is demonstrable in a significantly larger number of subjects than tricuspid regurgitation lending itself to the study of pulmonary haemodynamic changes in normals (Chan KL et al, 1987, Tramarin R et al, 1991). We will therefore confine ourselves to primarily describing the use of this technique and its limitations with some reference to the measurement of pulmonary artery pressure based on trans tricuspid gradients

Technical considerations

The normal pattern of pulmonary artery flow is a gradually accelerating then decelerating laminar flow peaking in mid systole. This profile extends for several centimetres distal to the valve. Inappropriate positioning of the sample volume particularly medial to this stream can result in alterations in this profile, in particular significant shortening of the pulmonary acceleration time (PAT), an increase in peak velocity and wide frequency dispersion with a profile similar to that seen in pulmonary hypertension (Pandis IP et al, 1986). This can be avoided by accurate positioning of the sample volume. It has been demonstrated that a reduction in pulmonary acceleration time and an increase in peak velocity occurs as the probe moves distally from the right ventricular outflow tract to the centre of the pulmonary artery (Pandis IP et al, 1986). Although some investigators have sampled from the right ventricular outflow tract (Tramarin R et al, 1991), because pulmonary artery flow varies with sample location our practice is to sample distal to the pulmonary valve allowing the optimal signal to be recorded more easily with an angle of incidence always less than 15° so minimising sampling errors (Gardin JM et al, 1984)

In patients with pulmonary hypertension the flow velocity pattern can be recorded in greater than 85% of patients (Chan KL et al, 1987, Kitabatake A et al, 1983). It is changed with a more rapid increase in velocity after valve opening, an earlier peak velocity and an earlier decrease in velocity although the peak velocity does not differ appreciably from normal. In addition a systolic notch is present in approximately 40-50% of patients and appears to be associated with more severe disease. In pulmonary hypertension shortening of the PAT has been shown to be the most consistent finding in these patients. Dabestani (Dabestani A et al, 1987) showed that a PAT of 100ms or less resulted in a 78% sensitivity and a 100% specificity for the detection of elevated pulmonary artery pressure which was corroborated by the work of Handshoe (Handshoe R et al, 1985) who showed that a PAT of less than 106ms had a sensitivity of 79% and specificity of 100% for an abnormal pulmonary artery pressure although other investigators have found predictive accuracy only when the PAT is less than 80ms which in itself has been shown to be a good predictor of three year mortality in cor pulmonale (Stevenson JG et al, 1989).

Doppler technique

Our measurements were made using a Vingmed SD50 which we have previously described. In addition a Vingmed SD 200 was used to screen all patients for underlying valvular disease and in addition to establish the position of the valves and outflow tracts to aid in positioning of the stand alone Doppler probe on the SD 50. Study subjects were selected because of technically optimal Doppler studies and a clearly defined spectral display of the pulmonary artery flow. The PAT was defined as

the interval from the onset of pulmonary flow to peak velocity. Normal volunteers were studied in a supine position rolled slightly on their left side. After initial interrogation of the echocardiographic/Doppler windows the 2.0 Mhz Doppler probe was angled and the sample depth adjusted until a clear envelope was obtained. Fine adjustments were then made to the sample depth to attain maximal pulmonary artery velocity whilst maintaining a clear Doppler signal. Measurements were then made on-line with a display speed of 100mm/s. Three complexes were then measured and averaged and this value was recorded as the measured PAT. The criteria for acceptance of an adequate signal were the presence of a whistling sound of lower frequency than for aortic flow when listening to the audible signal and a smooth velocity envelope. Repeated interrogation of the Doppler flow was then made from the same position at the same depth and angulation, as suggested by Dabestani (Dabestani A et al, 1983) when evaluating the effects of vasodilators, allowing for fine adjustments to be made in order to obtain the optimal signal. In normal volunteer studies the pulmonary artery flow measurements were made from 2/3/4 intercostal spaces parasternally and in patients with cor pulmonale the pulmonary valve was interrogated subcostally. MPAP was then calculated from the PAT using Dabestani's regression $MPAP = 73 - (0.42 \times PAT)$ for normal volunteer studies and $MPAP = 73 - (0.42 \times PAT)$ for our patient studies, all of whom had PAT's of less than 110ms (Dabestani A et al, 1987).

Validation and reproducibility of Doppler measures of pulmonary artery pressure

Good correlations have been found between PAT and catheter measures of MPAP (Dabestani A et al, 1987, Beard JT et al, 1991, Chan KL et al, 1987, Jiang L et al, 1984, Matsuda M et al, 1986, Kitabatake A et al, 1993, Kiely DG et al 1997a). Dabestani found a significant negative correlation between PAT and MPAP in a group of 39 patients (16 of whom did not have elevated pulmonary artery pressures) undergoing diagnostic cardiac catheterisation. The regression equation relating these parameters over the range of pulmonary artery pressures $MPAP = 73 - (0.42 \times PAT)$ had a correlation coefficient of $r = -0.84$; $p < 0.001$. In patients with a PAT of less than 120ms the regression equation linking these parameters $MPAP = 90 - (0.62 \times PAT)$ had a correlation coefficient $r = -0.87$. Similar correlations have been done by other investigators and indeed the work of our own group showed a good correlation between these PAT and MPAP over a range of pulmonary artery pressures with an correlation coefficient $r = -0.88$ (Kiely DG et al, 1997a).

To try and improve the correlation and reduce variation a number of ratios have been used including the ratio of PAT/right ventricular ejection time (RVET), the ratio of right ventricular pre-ejection period (RVPEP)/PAT and RVPEP/RVET. Although some studies have shown correlations similar to those using PAT (Dabestani A et al, 1987, Kitabatake A et al, 1983) several studies have shown that this ratio compares less favourably (Chan KL et al, 1987, Beard JT et al, 1991, Kosturakis D et al, 1984) and as a consequence we have used Dabestani's regression equations for the calculation of MPAP in our study. Our experience and certainly that of others is that

it is sometimes difficult to measure precisely termination of systolic flow and in addition at high pulmonary artery pressures , admittedly significantly higher than the great majority of our subjects, flow may terminate early, inappropriately shifting the ratio towards normal. Although some investigators have suggested that the correlation between PAT and MPAP can be improved by comparing PAT to \log_{10} MPAP (Kitabatake A et al, 1983) this has not been generally accepted (Chan KL et al, 1987, Chow LC et al, 1988).

It has been suggested that heart rate may affect PAT but this does not appear to be a problem unless heart rates exceed 120 bpm (Weyman AE, 1993). This was not anticipated to be the case in any of our studies no corrections for heart rate were made.

Although a good correlation has been obtained between PAT and catheter measured MPAP and studies have been performed using non-invasive methodology that concur with results of invasive studies Chow (Chow LC et al, 1988) examined the changes in MPAP and PAT in patients before and after pulmonary thromboendarterectomy and although statistically significant the correlation between changes in PAT and catheter measures of MPAP was weak ($r=-0.41$). Beard (Beard JT et al 1991) showed a good correlation between changes in the ratio of PAT/Right ventricular ejection time and changes in catheter MPAP ($r=-0.73$ in 11 subjects made hypoxic) and although the correlation was weaker for changes in PAT ($r=-0.55$) he found a significantly better correlation between absolute PAT and absolute MPAP than absolute PAT/Right ventricular ejection time and absolute MPAP ($r=-0.84$ vs $r=-0.35$). We have used

changes in PAT to assess the response of the pulmonary vascular bed to a variety of different vasodilators. The validity of our work is dependent on a change in pulmonary acceleration time being proportional to changes in MPAP. Although the correlation between absolute values is good and changes observed using this non-invasive methodology concur well with studies using invasive methodology more work is required to validate changes in Doppler echocardiographically measured PAT as a measure of response to vasodilators.

It is generally accepted that continuous wave Doppler echocardiography, which is capable of measuring high flow velocities provides the best method of diagnosis of pulmonary hypertension in a patient population (Tramarin R et al, 1991, Burghuber OC, 1996). It is a direct method whereas measures using pulmonary flow are based on correlation. The usefulness of this technique however, depends on the presence of tricuspid regurgitation and the ability to obtain accurate transducer measurements as well as the ability to accurately estimate right atrial pressure. Recent work has suggested that the accuracy of the latter can be improved using 2D echocardiographic measurements of atrial size in addition to colour Doppler flow measurements (Burghuber OC, 1996). This aside a good correlation has been obtained with systolic pulmonary artery pressure using this methodology with coefficients of variation ranging from 0.68 to 0.98 (Tramarin R et al, 1991, Himelman RB et al, 1989). A European multicentre study compared a variety of different techniques and concluded that a Doppler measure of pulmonary artery pressure could be obtained in 98% of patients with COPD (Tramarin R et al, 1991). In this study Doppler recordings of tricuspid regurgitation adequate for velocity measurements could only be obtained in

30% of patients overall and in only 11% of patients with normal pulmonary artery pressures compared to an analysable pulmonary flow velocity profile in 98% of cases and right ventricular isovolumic relaxation time in 61% of patients. All measures were found to be reproducible but the best correlation was found between pulmonary artery pressure measured by transtricuspid regurgitation ($r=0.73$) compared to PAT ($r=0.65$) and right ventricular isovolumic relaxation time ($r=0.61$). These correlation coefficients are generally lower than that reported in other studies and in this study may relate to a number of factors such as the large number of patients with normal pulmonary artery pressures (37%). It has been shown that the relationship between pulmonary flow velocities and catheter measures of MPAP is not linear over a wide range of pressures and that the correlation can be significantly improved by performing regression analysis on patients with for example PAT of less than 120ms (Dabestani A et al, 1987). The multicentre nature of this study is likely to have increased scattering around the lines of regression and importantly the regression equations were not based on simultaneously made measurements. This is important in view of the fact that spontaneous variability at rest in COPD patients can exhibit a coefficient of variability of approximately 10%.

Although other investigators have found that transtricuspid regurgitation can be measured in most patients with COPD and in large numbers of normal volunteers using colour flow mapping and techniques such as saline enhancement we have used pulmonary artery flow measurements in our series of studies because of the good correlation that has been found between PAT and MPAP by ourselves and others, the reproducibility of this measure and the ability to obtain good Doppler profiles in

significant numbers of both our patients and normal volunteers. Table 2.2 summaries the relationship between Doppler and catheter measures of pulmonary artery pressure including our own published data.

Key to Table 2.2

The correlation coefficient (r) for the linear regression equation linking the variables in column 2 is given along with the patient group in which the study was performed. Where referred to as “mixed patients” the data may include patients with a wide variety of cardiopulmonary conditions including primary pulmonary hypertension, ischaemic heart disease, valvular heart disease, congenital heart disease, cardiomyopathies and does not necessarily imply the presence of pulmonary hypertension. Indeed the majority of these studies recruited patients undergoing diagnostic cardiac catheterisation at which time Doppler echcardiography was performed. Where the patients are indicated as having COPD, the patient group contains exclusively these patients. PAT=pulmonary acceleration time; RVET=right ventricular ejection time, PEP=right ventricular pre-ejection period; TR=peak velocity of transtricuspid regurgitant jet; PSAP=pulmonary systolic arterial pressure; RVAT=right ventricular acceleration time; RVIVRT=right ventricular isovolumic relaxation time.

Table 2.2. Relationship between Doppler and catheter measures of pulmonary artery pressure

Dabestani et al	PAT vs MPAP	$r = -0.84$	n=39	mixed patients
	PAT vs log10 MPAP	$r = -0.84$	n=39	mixed patients
	PAT/RVET vs MPAP	$r = -0.80$	n=39	mixed patients
	PAT/RVET vs log10 MPAP	$r = -0.82$	n=39	mixed patients
	PAT vs MPAP	$r = -0.87$	n=27	patients, PAT < 120ms
Beard et al	PAT vs MPAP	$r = -0.84$	n=21	patients and normals
	PAT/RVET vs MPAP	$r = -0.35$	n=21	patients and normals
Chan et al	TR vs PSAP	$r = -0.87$	n=36	mixed patients
	PAT vs MPAP	$r = -0.85$	n=44	mixed patients
Jiang et al	PAT vs MPAP	$r = -0.86$	n=34	mixed patients
	PAT/RVET vs MPAP	$r = -0.86$	n=34	mixed patients
	PEP/PAT vs MPAP	$r = -0.94$	n=34	mixed patients
	PEP/RVET vs MPAP	$r = -0.68$	n=34	mixed patients
Matsuda et al	PAT vs MPAP	$r = -0.75$	n=67	mixed patients
Himelman et al	TR vs PSAP	$r = 0.98$	n=10	COPD
Kitbatake et al	PAT vs MPAP	$r = -0.82$	n=33	mixed patients
	PAT vs log10 MPAP	$r = -0.88$	n=33	mixed patients
Tramarin et al	RVAT vs MPAP	$r = -0.65$	n=97	COPD
	TR vs PASP	$r = 0.68$	n=30	COPD
	RVIVRT vs MPAP	$r = 0.61$	n=61	COPD
Kiely et al	PAT vs MPAP	$r = -0.88$	n=17	mixed patients

Our group has published reproducibility data in both normal man and also in patients with cor pulmonale that concurs well with that obtained in other studies. Our published coefficients of variability of MPAP calculated from PAT are quoted in the relevant chapters but range between 4-6% in normal healthy subjects and in patients with hypoxaemic cor pulmonale.

2.3.2 Total pulmonary vascular resistance

This was expressed in dyne.s.cm^{-5} as total pulmonary vascular resistance (TPR) = $\text{MPAP/CO} \times 80$. When interpreting changes in TPR as a reflection of changes in pulmonary vascular tone a limitation of this methodology is that TPR neglects the downstream pressure of the pulmonary circulation, namely left atrial pressure, as conventionally assessed by pulmonary capillary wedge pressure (PCWP). Although studies have shown that stimuli such as hypoxia and hypercapnia have no significant effects on PCWP this can obviously be affected by changes in ventricular function and this has been alluded to in the relevant parts of the discussion.

2.4 MEASURES OF SYSTOLIC AND DIASTOLIC FUNCTION

2.4.1 Systolic function

The non-invasive assessment of systolic ventricular function has become increasingly important with the advent of effective therapies for systolic heart failure. Although

assessments of systolic function can be made during cardiac catheterisation treatment in clinical practice is usually based on the results of an ejection fraction obtained from a multiple upgated acquisition scan or more commonly on the basis of a two dimensional echocardiogram. In this thesis we undertook to assess the cardiovascular effects of acute hypoxaemia and hypercapnia in normal man. To detect changes of systolic cardiovascular performance the methodology must be sensitive, reproducible and valid. A number of non-invasive measures of cardiovascular performance have been developed over recent years and used in clinical pharmacology as well as in clinical practice and include, two dimensional echocardiography, Doppler aortic blood flow measurements, systolic time intervals and transthoracic impedance cardiography. We have chosen systolic time intervals and Doppler aortic blood flow measurements because of their reproducibility and sensitivity in the assessment of changes in systolic function.

Technical considerations, validity and reproducibility of systolic time intervals

Systolic time intervals have been measured since the early 1920's and have been used extensively in a variety of different physiological studies allowing their limitations and strengths to be evaluated (Li Q et al, 1993). Investigators have focused their attentions primarily on three time intervals: total electromechanical systole (QS₂) measured from the onset of the QRS complex to aortic valve closure, the pre-ejection period (PEP) measured from the onset of ventricular depolarisation to the beginning of left ventricular ejection and representing isovolaemic contraction and finally left ventricular ejection time representing isotonic contraction and lasting from the onset to cessation of systolic aortic blood flow. Standardised conditions have been shown

to be important and if the studies are carried out under these controlled conditions excellent reproducibility has been demonstrated with Scott (Scott MJ et al, 1989) showing coefficients of variation for the same subjects on different study days ranging between 1.2 to 7.0 %, which is similar to the work of our own group with coefficients of variation for QS₂I 3.5% (Kiely DG et al, 1997b), LVET 1.6% (Lipworth BJ et al, 1994). STI's are dependent as are other measures of left ventricular function on heart rate and loading conditions. Although there appears to be some non-linearity between heart rate and STI's reflected by the linearity of STI's and beat to beat intervals (Wolf GK et al, 1978) these deviations are small and unlikely to affect results unless extreme extrapolations are used. We have therefore corrected our intervals according to standard criteria (Weissler AM et al, 1970). Recent studies have shown however that although the expected relationships existed between QS₂ and LVET and heart rate (Kelman AW et al, 1981) that there did not appear to be such a convincing relationship for PEP and heart rate (Spoddick DH et al, 1984). There is some debate therefore whether corrections should be used for this measure. We have therefore presented both corrected and uncorrected data where appropriate. Information regarding the effects of loading conditions on systolic time intervals is based on in vitro studies using isolated cardiac tissues where loading conditions can be closely controlled (Nakamura Y et al, 1983) and in vivo where one haemodynamic change can alter a number of haemodynamic variables. Positive inotropism shortens QS₂, PEP and LVET whereas negative inotropism prolongs these intervals (Li Q et al, 1993). Although changes in preload and afterload in vivo and using in vitro models affect both PEP and LVET the effects on QS₂ are less marked and this index is thought to reflect primarily inotropic effects with loading conditions having

significantly less effect (Nakamura Y et al, 1983, Lewis RP et al, 1977, Stern HC et al, 1984). These effects apart the STI have been shown to be sensitive measures of comparing drug induced changes in systolic function and in this respect de Mey and colleagues showed that in a dose ranging study using isoprotenerol that STI's were capable of detecting changes at the lowest dose whereas a 2 fold increase in this dose required to produce significant changes in aortic blood flow measured using Doppler echocardiography and impedance echocardiography whereas a fourfold increase in dose was required to produce significant changes as measured using two dimensional echocardiography (de Mey C et al, 1992)

Technical considerations, validity and reproducibility of Doppler measures of aortic blood flow

As alluded to above Doppler measures of aortic blood flow can be used to measure changes in systolic performance. Studies have been performed in both normal subjects and in patients with a variety of cardiac conditions using both pulsed wave and continuous wave Doppler echocardiography to assess left ventricular function at rest and after a number of different haemodynamic and pharmacological interventions. Noble (Noble MIM et al, 1966) has shown in a number of animal experiments that maximal aortic acceleration is a sensitive indicator of inotropic state and relatively insensitive to the loading conditions of the heart. Subsequent work has shown that blood acceleration was intrinsic to the rate of change in power developed by the left ventricle (Stein PD et al, 1976a) and a study in patients during cardiac catheterisation using catheter tip velocity sensors demonstrated that peak acceleration was capable of differentiating patients with normal from abnormal ventricular performance (Stein PD

et al, 1976b). The advent of Doppler echocardiography has allowed us to measure these indices non-invasively. Sabbah and colleagues (Sabbah NH et al, 1986) measured both peak aortic velocity and acceleration in 36 patients undergoing diagnostic cardiac catheterisation they found that both aortic peak acceleration ($r=0.90$) and peak aortic velocity ($r=0.77$) had good correlations with ejection fraction and that these indices were capable of distinguishing patients with low ejection fractions from those with normal left ventricular systolic function. It is known however that loading conditions can affect these indices (Bennet ED et al, 1984 Bedotto JB et al, 1989) and changes in response to haemodynamic stimuli must be interpreted with these potentially confounding effects taken into account.

The reproducibility intra and interobserver variation of Doppler aortic blood flow measurements were assessed by Gardin (Gardin JM et al, 1984) in 10 normal subjects over two visits. The intraobserver variability ranged from $1.9\pm1.8\%$ for ejection time to $3.2\pm2.9\%$ for peak aortic velocity to $7.9\%\pm6.6\%$ for acceleration time. Interobserver variability ranged from $3.5\pm2.2\%$ for ejection time to $5.4\pm3.4\%$ for peak aortic velocity to $17\pm9\%$ for acceleration time. Day to day variability was $3.6\pm3.9\%$ for ejection time, $5.2\pm4\%$ for peak aortic velocity and $7.0\pm5.2\%$ for acceleration time. They concluded that changes in aortic peak flow velocity, ejection time or flow velocity integral of greater than 13% or a change of aortic acceleration time of greater than 17% on serial recordings performed by the same technician would represent a true haemodynamic change. Hatle and colleagues (Hatle L et al, 1982) also reported reproducibility data in 5 normal subjects for 10 measurements made over a period of days to months and found a mean coefficient of variation of 6.4% for maximal

velocity and 8.6% for peak acceleration. The mean coefficient of variability for 5 measurements made in 16 subjects within minutes was 5.9% for peak velocity and 15.1% for peak acceleration which are similar to the results of our published work in 10 normal volunteers in whom 6 measurements were made over a period of 150 minutes; mean coefficients of variability 12.5% for mean acceleration and 18.7% for peak acceleration (Kiely DG et al, 1997b).

Doppler technique

Simultaneous lead II ECG, phonocardiogram (Siemens AG, Munich, Germany) and pulsed-wave Doppler ascending aortic blood flow (Vingmed SD50) were recorded. Doppler flow profiles were recorded with a non-imaging 2.0 MHz transducer with depth adjusted to give maximal velocity. All measurements were made with display sweep speed at 100 mm/sec and analysed in triplicate. From these recordings, the following variables were measured: Left ventricular ejection time (LVET) was measured as the time in ms from onset to end of systolic aortic flow, electromechanical systole (QS₂) was measured from the onset of the ECG Q wave to the second heart sound on the phonocardiogram and the pre-ejection period (PEP) as time in ms from ECG Q wave to onset of systolic aortic flow. QS₂, ET and PEP were also corrected for changes in HR according to standard criteria (Weissler AM et al, 1970), and are denoted QS_{2c} (or QS_{2I}), ET_c and PEP_c. Aortic peak acceleration (Acc_{peak}), aortic mean acceleration (Acc_{mean}), and aortic acceleration time (AT) as time in ms from onset to peak aortic flow where measured from ascending aortic blood flow.

2.4.2 Diastolic function

Diastole is a complex sequence of interrelated events involving myocardial relaxation, ventricular filling, atrial contraction, the viscoelastic properties of the myocardium, diastolic suction, pericardial constraint, ventricular interaction and the hydraulic effect of the coronary arteries (Clarkson PBM et al, 1994). Diastole encompasses the time period between aortic and mitral valve closure and can be divided into four intervals. The period of isovolumic relaxation (IVRT) during which time ventricular pressure rapidly declines without ventricular filling and extends from aortic valve closure to mitral valve opening. This phase is thought to be primarily attributable to active myocardial relaxation. It is followed by early filling due to the pressure gradient between the atria and ventricles which accounts for approximately 75% of filling at rest. The atrial and ventricular pressures then equalise and this phase accounting for approximately 5% of filling is a primarily passive process followed by atrial systole which contributes approximately 20% to filling in healthy young individuals.

Technical considerations and validation of Doppler echocardiography in the assessment of diastolic function

Assessment of diastolic function can be made by a number of different means. Presently much time is being spent trying to devise and validate a number of non-invasive measures, however the complexities of left ventricular filling mean that none of these are absolutely satisfactory. Invasive techniques allow measurement of continuous volume-time curves and pressure time curves allowing calculation of

indices of chamber rigidity and compliance and Tau-the time constant of relaxation (Weisfeldt M et al, 1974). Although radionuclide ventriculography has been shown to correlate well with invasive measures of diastolic function and is non-invasive (Seals A et al, 1986) its use is limited by its lack of precision and exposure to radiation. Echocardiography is now most frequently used in the assessment of ventricular diastolic function and several studies have been performed validating the use of Doppler measured transmitral flow profiles. Rokey and colleagues (Rokey R et al, 1985) compared Doppler transmitral flow profiles with cineangiography. They found a significant correlation between Doppler and angiographic peak filling rate ($r=0.87$), Doppler and normalised peak filling rate ($r=0.83$) and Doppler early peak diastolic velocity and angiographic peak filling rate ($r=0.64$). In addition there was a good correlation between early and late diastolic filling when Doppler and radionuclide angiography were compared (Spirito P et al, 1986). Studies have been performed comparing filling rates of transmitral Doppler recordings with indexes of diastolic function using invasive techniques such as measuring pressures using high fidelity micromanometers and volumes derived from cineangiography. Drinkovic and workers (Drinkovic N et al, 1986) did not find good correlations between Doppler measures and invasive indexes. Therefore although studies have shown that transmitral recordings provide a good representation of left ventricular filling rates they cannot be equated with filling rates calculated using conventional invasive indexes of diastolic function. Although not so well described Doppler measures of trans tricuspid filling also been used to evaluate right ventricular diastolic function (Marangoni S et al, 1992) where it has been shown to be useful in the diagnosis of this condition in chronic obstructive pulmonary disease.

We have experience with techniques for evaluating both right and left ventricular diastolic function using Doppler measured transmitral and transtricuspid filling rates and isovolumic relaxation times for both left and right ventricles. Our coefficients of variability for these measurements show that this methodology is reproducible. Published values (Cargill RI et al, 1995): mitral e-wave deceleration time (mitral EDT)=2.4%; tricuspid EDT=4.8%; right ventricular IVRT=12.2%; left ventricular IVRT=1.1%. Kiely (Kiely DG et al, 1997b) mitral E wave peak velocity E_{vmax} =12.2%; mitral A wave peak velocity A_{vmax} =17.2%; transmitral E/A ratio =12.4%; left ventricular IVRT 13.8%; tricuspid E_{vmaz} =8.8%; tricuspid A_{vmax} =14.2%; right ventricular IVRT=29.1%; transtricuspid E/A ratio=14.8%.

Doppler technique

From the apical window, pulsed-wave Doppler analysis of mitral and tricuspid diastolic flow (Vingmed SD50) was combined with simultaneous phonocardiogram recording with the microphone (Siemens AG; Munich, Germany) positioned over the 2nd left intercostal space. Measurements were all made on-line during expiration and in triplicate, with a display sweep speed of 100mm/sec. Transmitral and transtricuspid flow was analysed after adjusting sample volume depth to yield maximal E-wave velocities with clearly defined flow velocity envelopes. Aortic and pulmonary components of the second heart sound were identified on the phonocardiogram trace by noting closure artefacts from superimposition of aortic and pulmonary Doppler flow profiles. From diastolic transmitral and transtricuspid flow, maximal velocities of the early (E_{vmax}) and atrial (A_{vmax}) components of flow were measured, and the E/A ratio was calculated. In addition, the E wave deceleration time (EDT) was calculated as the time in milliseconds from peak velocity to the end of the E wave. The isovolumic relaxation time (IVRT) was calculated for the left ventricle as the time

in milliseconds from the aortic component of the phonocardiogram second heart sound to the onset of diastolic transmitral flow, and for the right ventricle, the time from the pulmonary component of the phonocardiogram second heart sound to the onset of diastolic transtricuspid flow. Both time intervals (EDT and IVRT) were also corrected for changes in heart rate induced by hypoxaemia by dividing by the square root of the simultaneous ECG R-R interval; $EDT_c = EDT/\sqrt{RR}$, $IVRT_c = IVRT/\sqrt{RR}$.

2.5 ELECTROPHYSIOLOGICAL INDICES

2.5.1 QT interval and QT dispersion

The surface electrocardiogram has been investigated for its ability to identify those patients at risk of arrhythmias and as such represents a cheap, non-invasive and simple method. Traditionally the single measurement of QTc interval has been used to measure the recovery of ventricular excitability, widely recognised to be the most important factor in arrhythmogenesis.

QT prolongation has been shown to be a predictor of sudden death in alcoholic cirrhosis (Day CP et al, 1993), sudden cardiovascular mortality in apparently healthy individuals (Schouten EG et al, 1991), as well as sudden death in patients with ischaemic heart disease (Puddu PE et al, 1986). Recently however, QTc dispersion (interlead variability in QTc interval) has been suggested as a more sensitive and specific marker as it measures differences in regional repolarisation and has been shown to be increased in patients with hypertrophic obstructive cardiomyopathy at risk from ventricular arrhythmias (Buja G et al, 1993) and in patients with long QT intervals it distinguished between those with ventricular arrhythmias and those without

(Day CP et al, 1990). Recently it has been found to be a more sensitive and specific marker of sudden death in heart failure than QTc interval alone (Barr CS et al, 1994).

To measure QT dispersion we used a computer linked digitising tablet which has been shown by other investigators to be a reliable and accurate measure of QTc dispersion (Bhullar HK et al, 1993). Probably the most important aspect concerning methodology is the protocol to define the end of the T wave. We have thus used the most commonly used protocol (Higham PD et al, 1994) and one which has been shown to correlate with arrhythmia risk and sudden death. We also measured the QTc interval in the majority of leads in each individual although occasionally leads were omitted due to difficulty defining the end of the T-wave. We used routine ECG's to measure QTc dispersion since we felt this would have most clinical relevance and indeed no substantial evidence suggests that simultaneous ECG recording has any benefits.

2.5.2 QT Interval and QT dispersion measurement

The ECG's from the study days were analysed in random order after completion of the study, by an investigator who was blinded with respect to which was the pre-post intervention ECG. QT interval if feasible was measured in all leads of a surface 12 lead ECG (paper speed = 25mm/s). Three consecutive cycles were measured in each lead where possible and the mean value was taken as representing the QT interval in that lead. QT interval was calculated according to standard criteria (Higham PD et al, 1994) from the onset of the QRS complex to the end of the T wave ie a return to the T/P baseline. In the presence of U waves the QT interval was measured to the nadir of the curve between the T and the U waves. QT intervals were then corrected for rate using Bazett's formula (Bazett HC, 1920) ($QT_c = QT/\sqrt{RR}$).

QT dispersion was defined as the difference between the maximum and minimum QT interval measured during analysis of all leads of the surface ECG whereas QTc dispersion was defined as the difference between the maximum and minimum QTc interval (Higham PD et al, 1994). The measurements were made using a computer linked digitising tablet (Bhullar HK et al, 1993). To compare the standard measure of QTc interval with QTc dispersion we calculated the average of three QT intervals in lead II and corrected for heart rate using Bazett's formula ($QTc = QT/\sqrt{RR}$).

2.6 NEUROENDOCRINE VARIABLES

2.6.1 Sampling

Blood samples for measurement of resting neurohormonal hormones and plasma electrolytes and serum creatinine was made after the subject or patient had rested in the supine position for at least 30 minutes to achieve baseline haemodynamic parameters. Blood was sampled from an intravenous cannula inserted into the antecubital fossa which was flushed with saline to keep it patent. After removal of 5ml of dead space blood from the cannula which was discarded blood was then taken for analysis. Further samples were then taken in accordance with the study protocol.

The only exception to the above was in the study entitled "A management role for natriuretic peptides in the diagnosis of pulmonary thromboembolism?" where venous blood samples were taken from patients in the supine position at the time of the

perfusion scan, prior to injection of Tc labelled macroaggregated albumin. Patients were required to have rested supine for at least 15 minutes.

2.6.2 Endothelin-1

Samples were collected into chilled EDTA tubes and then centrifuged at 2000 rpm at 4°C for 15 minutes and stored at -70°C until assayed in a batch on completion of the study. Assay was carried out in duplicate using a commercially available radioimmunoassay (RIA) kit (Nicholls Institute Diagnostics, San Juan Capistrano, CA, USA) after solid phase extraction on C18 silica columns. The specificity of the Nichols Institute Diagnostics Endothelin RIA was determined by cross reactivity of several compounds. The anti-endothelin antibody used had cross-reactivity of 100% with endothelin-1, 96% with endothelin-3 and 7% with proendothelin. Cross reactivity with ANP, angiotensin II and ACTH was less than 0.1%. The lower limit of detection was 0.4 pmol/l and the intra-assay co-efficient of variation was 4.5% and the interassay coefficient of variation 6.8%.

2.6.3 Natriuretic peptide system

Samples were collected into chilled EDTA tubes containing 4,000 kIU of aprotonin. Plasma was separated by centrifugation at 3,000 rpm for 15 minutes at 4C and stored at -70C until measurement of ANP-like, BNP-like and N-ANP-like immunoreactivity in one batch at the end of the study. After solid phase extraction from plasma, assays were performed in duplicate using commercially available radioimmunoassay kits (Penninsula Laboratories, St Helens, United Kingdom). The coefficients of variability

for each assay were: ANP intra-assay = 12.6%, interassay = 11.8%; BNP intra-assay = 9.9%, interassay = 14.8%; N-ANP intra-assay = 7.7%, interassay = 11.7%.

2.6.4 Renin angiotensin aldosterone system

Samples for measurement of plasma renin activity (PRA) were collected into chilled EDTA tubes and samples for plasma aldosterone were collected in chilled lithium-heparin tubes. They were centrifuged at 4°C immediately and separated plasma was stored at -20°C until assayed in one batch in duplicate at the end of the study. PRA and plasma aldosterone were assayed using commercially available RIA kits (Sorin Biomedica, Saluggia, Italy) . PRA was assayed by measurement of amount of angiotensin I generated per hour. Samples for ANG II assay were collected into chilled glass tubes containing 0.5 ml of a cocktail comprising 0.05mmol/l, 0-phenanthroline, 0.2g neomycin, 0.125 mmol/l EDTA and 2% (v/v) ethanol before centrifugation and separated plasma was stored at -70°C. ANG II assay was performed following separation from plasma proteins by ethanol extraction using a specific commercially available RIA kit (Nichols Institute Diagnostics, Juan Capistrano, CA, USA). The intra-assay coefficients of variability for each assay were: PRA = 6.50% and plasma aldosterone = 8.77%.

2.6.5 Catecholamines

Samples for adrenaline and noradrenaline were collected in chilled lithium-heparin tubes and were centrifuged at 4°C immediately. Separated plasma was stored at -70°C and assayed in one batch at the end of the study using the double isotope radioenzymatic method (Brown MJ et al, 1980).

2.7 SERUM ELECTROLYTES

Samples for serum electrolytes were collected in chilled lithium-heparin tubes and were centrifuged at 4°C immediately and separated plasma was stored at -20°C until measured in duplicate in one batch at the end of the study using an internal caesium standard flame photometer (Instrumentation Laboratory, Milan, Italy).

2.8 MEASUREMENT OF OXYGEN SATURATION AND END-TIDAL CARBON DIOXIDE CONCENTRATION

2.8.1 Oxygenation

Arterial blood oxygen saturation was continuously monitored by transcutaneous oximetry with the probe placed on the index finger (CSI 503, Criticare Systems Inc, Waukesha, WI, USA). When required for the purpose of analysis recordings were averaged at steady state over a period of 5 minutes at each time point.

2.8.2 End-tidal carbon dioxide concentration

This was measured continuously with the tip of the gas sampling tube adjacent to the mouth of the subject, using a transportable ET CO₂ monitor (POET TE, Criticare Systems Inc, Waukesha, WI, USA). End-tidal CO₂ is known to closely mirror the concentration of carbon dioxide in the blood. The ET CO₂ is approximately 0.5 Kpa lower than that measured in arterial blood. This difference is due to blood leaving the ventilated alveoli mixing with blood from both parenchymal lung tissue and with blood passing through non-ventilated alveoli so creating a venous admixture. It is this venous admixture that accounts for the normal alveolar-arterial carbon dioxide tension difference. When required for the purpose of analysis recordings were averaged at steady state over a period of 5 minutes at each time point.

CHAPTER 3

**LEFT VENTRICULAR SYSTOLIC PERFORMANCE
DURING ACUTE HYPOXAEMIA
IN NORMAL MAN**

Chest 1995;108:899-902.

(Editorial. The Heart in oxygen debt.

Chest 1995;108:889-891)

3.1 SUMMARY

Although some of the cardiovascular responses to hypoxaemia are well described, effects on myocardial contractility have not been defined. Such effects are readily assessed by non-invasive techniques and we have therefore evaluated Doppler-phonocardiographic parameters of systolic left ventricular contractility in normal humans rendered hypoxaemic.

Eight healthy male volunteers were studied. Parameters were measured after resting to achieve baseline haemodynamics, after 20 minutes moderate hypoxaemia (SaO_2 85-90%) and after a further 20 minutes of severe hypoxaemia (SaO_2 75-80%). Hypoxaemia was induced by breathing a variable N_2/O_2 mixture.

Pulsed-wave Doppler analysis of ascending aortic blood flow was combined with phonocardiography to measure indices of systolic left ventricular function at baseline and at the end of each period of hypoxaemia.

There was a significant, dose related increase in cardiac output in response to hypoxaemia, from 5.5 ± 0.26 l/min at baseline to 6.1 ± 0.08 l/min during moderate hypoxaemia and to 7.0 ± 0.23 l/min during severe hypoxaemia. Likewise, heart rate increased significantly in dose related fashion although stroke volume was not affected by either level of hypoxaemia. Hypoxaemia had no significant effects on systolic or diastolic blood pressures, but caused a significant reduction in systemic vascular resistance. Aortic peak and mean acceleration and acceleration time were not affected by moderate or severe hypoxaemia. Although the systolic time intervals measured shortened significantly during severe hypoxaemia these were no longer significant when appropriate corrections were made for heart rate.

Although cardiac output increases during hypoxaemia, this is due to increases in heart rate but not to any effect on stroke volume. Parameters of left ventricular systolic function and myocardial inotropic state were also not affected by severe hypoxaemia. Systolic left ventricular function and myocardial contractility is therefore well preserved in normal humans during hypoxaemia.

3.2 INTRODUCTION

Acute hypoxaemia commonly occurs in humans as a result of cardiorespiratory disease or adverse environmental conditions. The cardiovascular responses to the stress of hypoxaemia have therefore been of great interest. It is clear that acute hypoxaemia induces pulmonary hypertension due to hypoxic pulmonary vasoconstriction (Cutai M et al, 1990), increases heart rate and cardiac output (Phillips BA et al, 1988) without affecting systemic blood pressure (Cutai M et al, 1990, Vonmoos S et al, 1990). There is however no information available regarding the effects of hypoxaemia on intrinsic myocardial contractility during systole. Such effects may well be important in the assessment of cardiovascular performance in subjects with fluctuating levels of oxygenation.

The non-invasive assessment of systolic ventricular function has become particularly important with the advent of effective therapies for systolic heart failure (Consensus Trial Study Group, 1987). Although quantification of ejection fraction appears a suitable measure of systolic function, this is subject to other haemodynamic influences (Ross J Jr et al, 1979) and does not directly assess intrinsic myocardial contractility. This latter component can however be assessed by a number of other non-invasive methods including echocardiography and phonocardiography. Echo-Doppler assessment of aortic flow acceleration parameters have been used to study changes in overall ventricular performance and compares favourably with invasive measurements (Sabbah HN et al, 1986). Other systolic time intervals, derived in conjunction with phonocardiography have also been proposed to reflect the inotropic state of the left ventricle (Li Q et al, 1993) and have been used extensively to monitor drug induced changes in myocardial contractility (Belz GG et al, 1992, Belz GG et al, 1978).

We have therefore combined these two methods to assess for the first time the effects of moderate and severe acute hypoxaemia on parameters of systolic myocardial performance in humans.

3.3 METHODS

Subjects

Eight normal male volunteers, age (mean \pm SEM) 29.3 \pm 2.9 years were evaluated after giving informed written consent to the study protocol, previously approved by the Tayside Committee for Medical Research Ethics. All subjects had normal clinical history and examination, biochemical and haematological screening and 12 lead electrocardiogram.

Study design

All subjects attended the clinical laboratory at the same time of day and remained in a supine position throughout the study. Once stable resting haemodynamic parameters were obtained, subjects were rendered hypoxaemic by breathing a variable mixture of oxygen and nitrogen to achieve moderate hypoxaemia with SaO₂ between 85 and 90% for 20 minutes followed by 20 minutes of severe hypoxaemia with SaO₂ between 75 and 80%. Parameters were recorded at baseline and at the end of each 20 minute period of hypoxaemia.

Measurements

Systemic haemodynamic variables and systolic flow parameters were measured as previously described

Data analysis

Comparisons were made by multifactorial analysis of variance and where this was significant, Duncan's multiple-range testing was used to determine where significant differences lay. A probability value of $p < 0.05$ (two-tailed) was considered to be statistically significant.

3.4 RESULTS

Systemic haemodynamic changes.

Moderate hypoxaemia significantly increased CO by 0.7 l/min (95% confidence interval 0.1, 1.3) and HR by 4.5 beats/min (95% CI -0.7, 9.7) from baseline. Severe hypoxaemia caused further increases in CO; 1.5 l/min (95% CI 0.9, 2.1) and HR; 10.5 beats/min (95% CI 5.3, 15.7) (Table 1). SV however, was not affected by hypoxaemia, whilst SBP and DBP were unchanged during moderate or severe hypoxaemia (Table 3.1). SVR decreased significantly during moderate hypoxaemia (-138 dyne.s.cm⁻⁵ from baseline; 95% CI -2, -274) and during mild hypoxaemia (-245 dyne.s.cm⁻⁵ from baseline; 95% CI -109, -381) (Table 3.1).

Systolic flow parameters.

Acc_{peak} was similar at baseline (23.8±1.5 ms⁻¹) and after moderate (22.1±1.0 ms⁻¹) and severe hypoxaemia (21.4±2.5 ms⁻¹). Similarly, Acc_{mean} did not change from baseline (10.9±1.0 ms⁻¹) during either moderate (10.4±1.0 ms⁻¹) or severe hypoxaemia (12.0±1.8 ms⁻¹). AT was also unchanged from baseline (76±6.7 ms) during either moderate (75±6.5 ms) or severe hypoxaemia (73±6.7 ms).

Although similar at baseline (411±8 ms) and during moderate hypoxaemia (410±8 ms), QS₂ was significantly shortened during severe hypoxaemia (385±11 ms). Likewise ET was similar at baseline (293±9 ms) and during moderate hypoxaemia (289±10 ms) but shortened significantly during severe hypoxaemia (277±10 ms). PEP was also significantly shorter during severe hypoxaemia (109±8 ms) than at baseline (118±9 ms) or during moderate hypoxaemia (121±8 ms). However, following correction of these parameters for HR changes, neither QS_{2c}, ET_c or PEP_c were significantly affected by either level of hypoxaemia (Figure 3.1).

TABLES

Table 3.1. Systemic haemodynamic effects of acute hypoxaemia in normal man.

	Baseline	Moderate hypoxaemia	Severe hypoxaemia
HR(beats/min)	60±2.7	65±2.8*	71±4.3*†
SV(ml)	102±7.5	102±4.7	104±4.9
CO(L/min)	5.5±0.26	6.1±0.08*	7.0±0.23*†
SBP(mmHg)	120±7.5	125±6.8	125±6.9
DBP(mmHg)	65±3.8	70±3.7	67±4.0
SVR(dyne.s.cm ⁻⁵)	1224±80	1085±36*	979±59*

Absolute values (mean±SEM) of heart rate (HR), stroke volume (SV), cardiac output (CO), systolic blood pressure (SBP), diastolic blood pressure (DBP) and systemic vascular resistance (SVR) at baseline and during moderate and severe hypoxaemia.

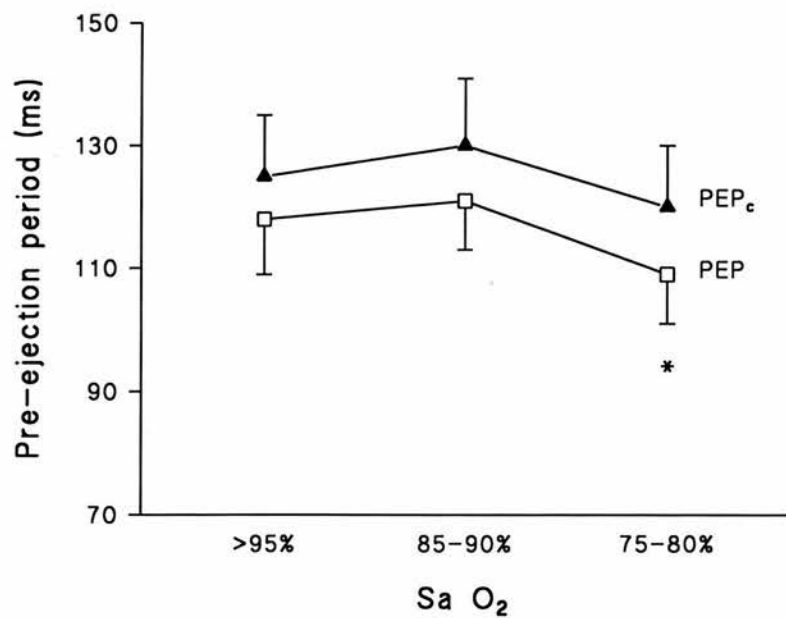
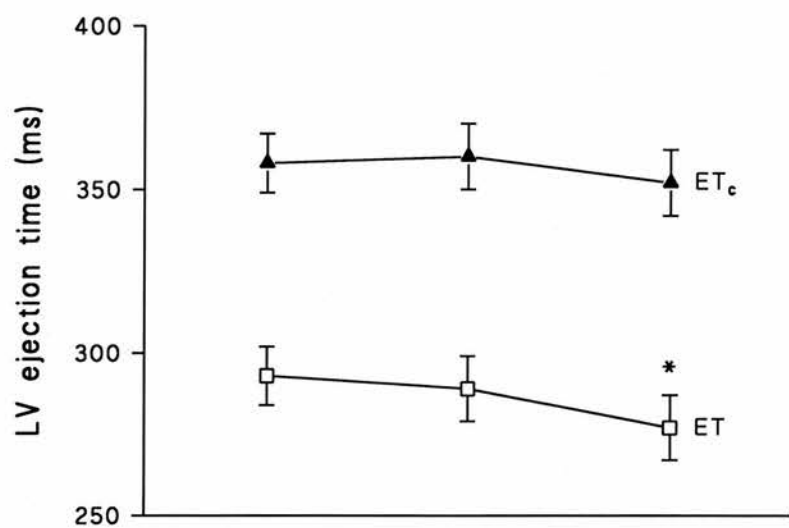
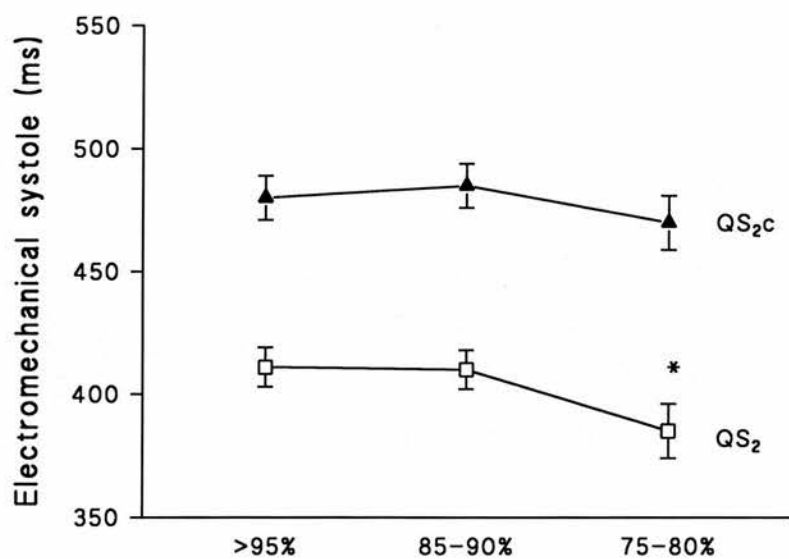
* significantly different from baseline

† significantly different from moderate hypoxaemia

FIGURE LEGEND

Figure 3.1. Effects of acute hypoxaemia on systolic flow indices and electromechanical systole in normal man.

Systolic time intervals, uncorrected corrected for changes in heart rate (c), showing QS₂, left ventricular ejection time (ET) and pre-ejection period (PEP) at baseline and during moderate and severe hypoxaemia.



3.5 DISCUSSION

We have shown that whilst causing significant changes in haemodynamic status, hypoxaemia does not impair left ventricular myocardial contractility in normal humans. It is therefore appropriate to consider these haemodynamic effects separately from indices of myocardial contractility.

These observations are in agreement with the work of other investigators (Phillips BA et al, 1988) who also noted using similar methodology, that increases in CO during hypoxaemia were the result of positive chronotropic effects rather than any effect on SV. The pulsed-wave Doppler techniques we have employed are however more accurate and reproducible (Coats AJS, 1990), comparing favourably with invasive measurement of CO (Labovitz AJ et al, 1985, Schuster AH et al, 1984). Unlike the work of Phillips et al (Phillips BA et al, 1988) we did not control PaCO₂ in our subjects. However, in view of the concordant findings it is unlikely that this has a bearing on CO over the SaO₂ range studied.

We also calculated SVR which fell significantly during hypoxaemia. The consequent decrease in left ventricular afterload by itself might therefore be expected to cause an increase in SV. Since SV did not change during hypoxaemia, this may have been limited by the increased right ventricular afterload secondary to hypoxic pulmonary vasoconstriction. The constant SV we observed would therefore make it less likely that mechanical factors in accordance with Starling's law (i.e. left ventricular distension) could have affected systolic left ventricular contractility during hypoxaemia.

Of the Doppler aortic acceleration indices, both mean and peak acceleration have been used as markers of left ventricular contractility (Bedotto JB et al, 1989) although Acc_{peak} appears to be relatively less influenced by changes in loading conditions

(Bennett ED et al, 1984). Furthermore, although Acc_{peak} declines with increasing HR during pacing (Harrison MR et al, 1989), the effects across the HR range of our study are small and consistent with the non-significant decrease observed. Thus, hypoxaemia has no significant effects on left ventricular contractility as measured by either peak or mean aortic acceleration.

The systolic time intervals are easily measured and repeatable markers of the inotropic state of the left ventricle (Li Q et al, 1993), which are in some cases more sensitive than echo-Doppler parameters (de Mey et al, 1992). Their changes with heart rate are well established and corrections appropriate to the study population are easily applied (Warrington SJ et al, 1988). As measures of the inotropic state of the myocardium, the systolic time intervals are prolonged if contractility is impaired with the QS_2 interval being relatively unaffected by changes in loading conditions (Li Q et al, 1993). We found that hypoxaemia did not affect heart rate corrected QS_2 , as has been found previously (Bremner P et al, 1992), or any of the other systolic time intervals measured. We can therefore be confident that hypoxaemia did not significantly alter left ventricular myocardial contractility.

It would appear therefore that systolic contractility of normal human myocardium is relatively resistant to the effects of hypoxaemia. This is in contrast to the effects of myocardial ischaemia where in addition to myocyte hypoxia, limitation of blood flow might also allow accumulation of anaerobic metabolites which impair contractility (Kilhara Y et al, 1989). These findings may well be important in determining the relevance of hypoxaemia as a cause of abnormal cardiovascular performance in hypoxaemic patients as well as in assessing haemodynamic responses to changes in oxygenation status.

CHAPTER 4

**ADVERSE EFFECTS OF HYPOXAEMIA
ON DIASTOLIC FILLING
IN NORMAL MAN**

Clinical Science 1995;89;165-169.

4.1 SUMMARY

Abnormalities of myocardial relaxation may occur as a consequence of myocyte hypoxia. We have therefore examined the effects of hypoxaemia on right and left ventricular diastolic function in 10 healthy male volunteers.

After resting to reach baseline haemodynamics, subjects were rendered hypoxaemic by breathing a variable nitrogen/oxygen mixture. Oxygen saturation (SaO_2) was maintained at 85-90% for 20 minutes and then 75%-80% for a further 20 minutes. Haemodynamic and diastolic filling parameters were measured noninvasively at baseline and at the end of each period of hypoxaemia.

Diastolic filling of both ventricles was significantly impaired by hypoxaemia. In comparison with baseline, left ventricular isovolumic relaxation time and transmitral E-wave deceleration time corrected for heart rate (IVRTc and EDTc) , were significantly prolonged at SaO_2 75-80%; IVRTc mean difference 9.8ms (95% confidence interval 1 - 19), EDTc mean difference 34ms (95% CI 11 - 56). Similarly, right ventricular IVRTc and transtricuspid EDTc were significantly prolonged at SaO_2 of 75-80% compared with baseline; IVRTc mean difference 20.3 ms (95% CI 3 - 38), EDTc mean difference 33 ms (95% CI 11 - 55) During hypoxaemia there were dose related increases in heart rate, cardiac output and mean pulmonary artery pressure, but no effects on mean arterial pressure.

Hypoxaemia significantly impairs relaxation of left and right ventricles in normal man. These changes may reflect impairment of intracellular calcium transport secondary to the effects of myocyte hypoxia.

4.2 INTRODUCTION

Ventricular diastolic function has emerged as an important determinant of overall cardiovascular performance (Clarkson PBM et al, 1994) and is a particularly sensitive marker of cardiac dysfunction. Abnormal diastolic function precedes systolic function abnormalities in a number of disease states, including ischaemic heart disease (Wind BE et al, 1987), hypertrophic cardiomyopathy (Pearson AC et al, 1987) and hypertensive left ventricular hypertrophy (Hatle L, 1993), and may contribute to the morbidity of these conditions.

Although most human studies of diastolic function have evaluated filling patterns of the left ventricle, the role of ventricular interaction during diastole (Janicki JS et al, 1980, Stojnic et al, 1992) might indicate the need to consider abnormalities of right and left ventricular filling together. In this respect, right as well as left ventricular filling is impaired in patients with ischaemic heart disease (Fujii J et al, 1985) and restrictive cardiomyopathy (Appleton CP et al, 1988) whilst patients with pulmonary hypertension have paradoxical impairment of left ventricular filling (Stojnic BB et al, 1992).

In many of the above conditions, it is difficult to differentiate between structural cardiac changes (e.g. ventricular remodelling) and functional abnormalities related to changes in myocyte metabolism. Cellular hypoxia and the consequent abnormalities of intracellular calcium transport (Kihara Y et al, 1989) may impair myocyte relaxation and hence may worsen overall diastolic function. These cellular events may be of relevance during myocardial ischaemia which leads to local hypoxia, or if hypoxaemia accompanies cardiopulmonary disease as in cor pulmonale where at least right ventricular diastolic function is impaired (Marangoni S et al, 1992).

To elucidate further the role of these functional abnormalities during diastole, we have studied the effects of hypoxaemia in normal humans. In this setting structural abnormalities can be excluded, allowing us to study in vivo the functional effects of hypoxaemia on left and right ventricular diastolic filling in man.

4.3 METHODS

Subjects

Ten normal male volunteers, age (mean \pm SEM) 28.0 \pm 1.9 years were evaluated. All subjects had no abnormality on clinical history and examination, biochemical and haematological screening or 12 lead electrocardiogram. In addition, echocardiographic study was required to be normal with high quality transmitral and transtricuspid Doppler flow profiles. Informed written consent to the study protocol, previously approved by the Tayside Committee for Medical Research Ethics, was obtained.

Study design

Subjects attended the clinical laboratory between 2 and 4 hours after lunch and remained in a supine position, rolled slightly on to the left side. After resting to obtain stable resting haemodynamic parameters, subjects were rendered hypoxaemic by breathing a variable mixture of nitrogen and oxygen to achieve an SaO₂ between 85 and 90% for 20 minutes followed by 20 minutes with SaO₂ between 75 and 80%. Haemodynamic variables and diastolic filling parameters were recorded at baseline and at the end of each period of hypoxaemia at steady state.

Measurements

Haemodynamic and diastolic filling indices were measured as previously described. In our hands the short term co-efficients of variation were as follows: mitral EDT 2.4%; tricuspid EDT 4.8%; left ventricular IVRT 1.1% and right ventricular IVRT 12.2%.

Data analysis

Comparisons were made by multifactorial analysis of variance and where this was significant, Duncan's multiple-range testing was used to determine where significant differences lay. A probability value of $p < 0.05$ (two-tailed) was considered to be statistically significant. Data are presented in the text and figures as means and standard error of the mean, and where a difference between means is quoted, the 95% confidence interval for this difference is also given.

RESULTS

Haemodynamic changes.

Hypoxaemia caused significant dose related increases in both HR and CO (Table 4.1). Hypoxic pulmonary vasoconstriction resulted in an increase in MPAP at both levels of hypoxaemia whilst no significant effects on systemic MAP were observed (Table 4.1)

Diastolic filling parameters.

Transmitral E/A ratio decreased progressively in response to increasing levels of hypoxaemia (Figure 4.1), due to significant increases in A wave velocity whereas E wave velocity was unchanged (Table 4.2). A similar pattern was observed for transtricuspid flow where reductions in the E/A ratio in response to hypoxaemia (Figure 4.2) were due to increases in Av_{max} rather than changes in Ev_{max} (Table 4.3).

Absolute levels of left ventricular IVRT were not significantly affected by hypoxaemia (Table 4.2) although after correction for changes in HR, IVRTc was significantly prolonged from baseline when SaO_2 was 85-90% (mean difference 8.9ms; 95% CI 1-16) and when SaO_2 was 75-80% (mean difference 9.8ms; 95% CI 1-19) (Figure 4.1). In the right ventricle, IVRT was prolonged when SaO_2 was reduced to 75-80% but not at 85-90% (Table 3). In comparison with baseline, right ventricular IVRTc was prolonged by hypoxaemia at SaO_2 75-80% (mean difference 20.3ms; 95% CI 3-38) but not at SaO_2 85-90% (Figure 4.2).

Mitral EDT was only prolonged at SaO_2 75-80% (Table 4.2). After correction for HR, EDTc was also significantly prolonged from baseline at SaO_2 75-80% (mean difference 34ms; 95% CI 11-57) (Figure 4.1). Tricuspid EDT was similarly prolonged by hypoxaemia (Table 4.3) and tricuspid EDTc was significantly prolonged from baseline only at SaO_2 75-80% (mean difference 33ms; 95% CI 11-55) (Figure 4.2).

Comparison of changes between left and right ventricles showed that as a percentage of baseline IVRTc, at SaO₂ 85-90% right ventricular IVRTc increased by 51% compared with an increase in left ventricular IVRTc of 13% (mean difference 38%; 95% CI -11-100. p=ns). At the higher level of hypoxaemia where SaO₂ was reduced to 75-80%, right ventricular IVRTc increased by 72% compared with an increase in left ventricular IVRTc of 14% (mean difference 58%; 95% CI 6-109. P<0.05).

Table 4.1. Haemodynamic effects of acute hypoxaemia in normal man.

	SaO ₂		
	>95%	85-90%	75-80%
HR(beats/min)	62.1±2.9	67.9±3.5*	74.5±4.5*†
CO(L/min)	5.59 ± 0.28	6.41± 0.41*	7.22± 0.42*†
MAP(mmHg)	85.0±3.4	87.2±3.3	88.1±3.6
MPAP(mmHg)	9.3±0.4	17.8±0.8*	27.6±1.0*†

Absolute values (mean±SEM) of heart rate (HR), cardiac output (CO), mean arterial pressure (MAP) and mean pulmonary artery pressure (MPAP) at each level of oxygenation.

* significantly different from >95%

† significantly different from 85-90%

Table 4.2. Effects of acute hypoxaemia on left ventricular diastolic filling parameters in normal man

	SaO ₂		
	>95%	85-90%	75-80%
Ev_{max}(m/s)	0.70±0.02	0.69±0.02	0.71±0.04
Av_{max}(m/s)	0.44±0.01	0.48±0.02*	0.57±0.03*
E/A	1.53±0.03	1.44±0.06*	1.24±0.02*†
IVRT(ms)	70.7±6.0	74.8±6.5	72.5±7.1
EDT(ms)	146±5.6	150±8.3	168±14.4*

Absolute values (mean±SEM) of maximal transmitral early (Ev_{max}) and atrial (Av_{max}) wave velocity, E/A ratio, left ventricular isovolumic relaxation time (IVRT) and E wave deceleration time (EDT) at each level of oxygenation.

* significantly different from >95%

† significantly different from 85-90%

Table 4.3. Effects of hypoxaemia on right ventricular diastolic filling parameters in normal man.

	SaO₂		
	>95%	85-90%	75-80%
Ev_{max}(m/s)	0.57±0.02	0.61±0.03	0.61±0.05
Av_{max}(m/s)	0.36±0.01	0.44±0.03*	0.50±0.04*
E/A	1.57±0.06	1.41±0.08*	1.22±0.07*†
IVRT(ms)	40.1±4.3	50.0±7.6	52.9±8.9*
EDT(ms)	150±6.1	151±7.0	170±8.3*

Absolute values (mean±SEM) of maximal transtricuspid early (Ev_{max}) and atrial (Av_{max}) wave velocity, E/A ratio, right ventricular isovolumic relaxation time (IVRT) and E wave deceleration time (EDT) at each level of oxygenation.

* significantly different from >95%

† significantly different from 85-90%

FIGURE LEGEND

Figure 4.1a, b and c.

Parameters of left ventricular diastolic function showing a) E/A velocity ratio, b) heart rate corrected isovolumic relaxation time (IVRTc) and c) heart rate corrected E-wave deceleration time (EDTc). Values are means and SEM.

* denotes significant difference from baseline ($\text{SaO}_2 > 95\%$)

⁺ denotes significant difference from SaO_2 85-90%

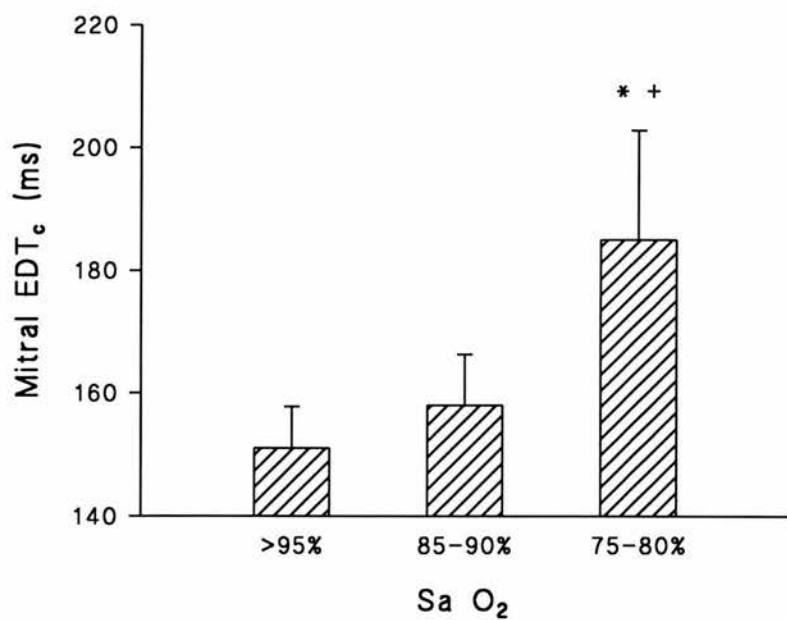
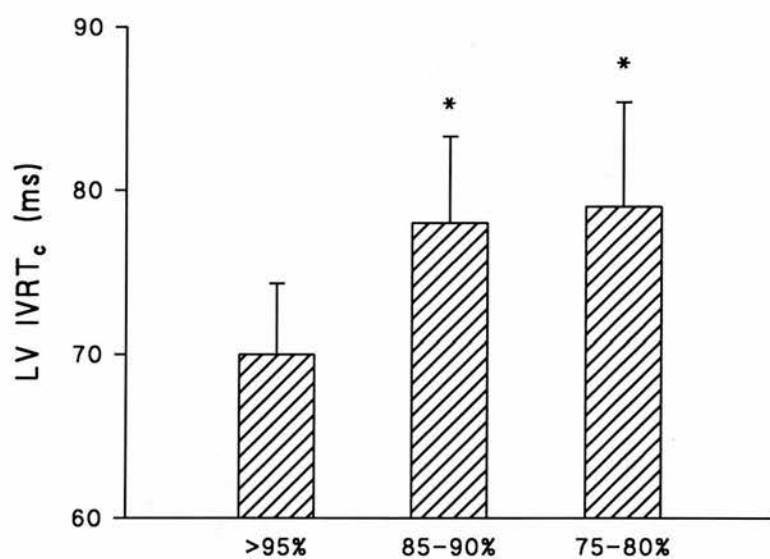
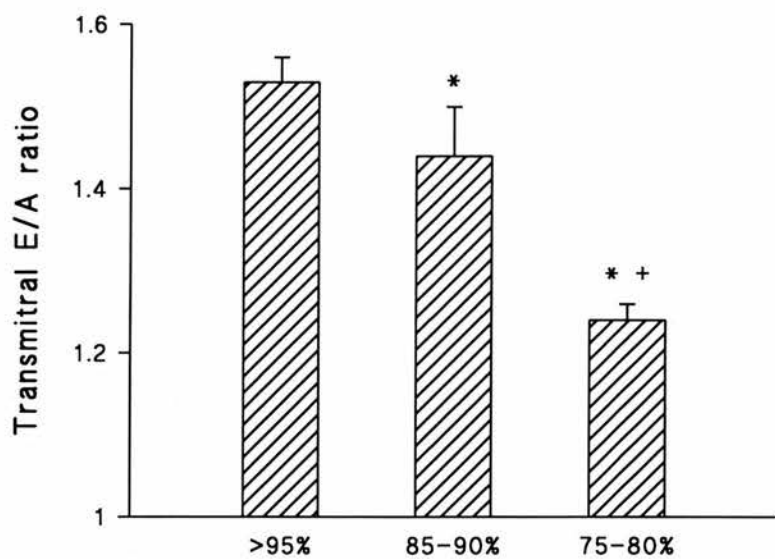


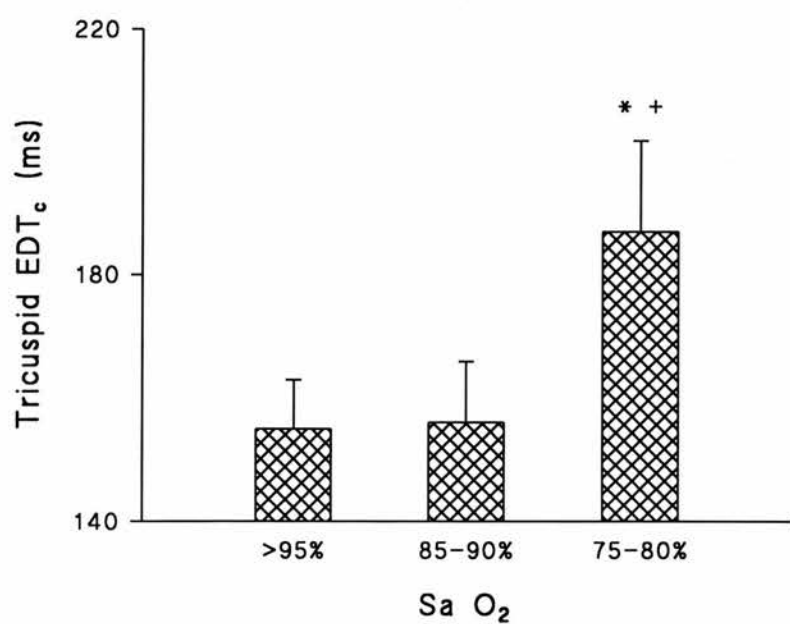
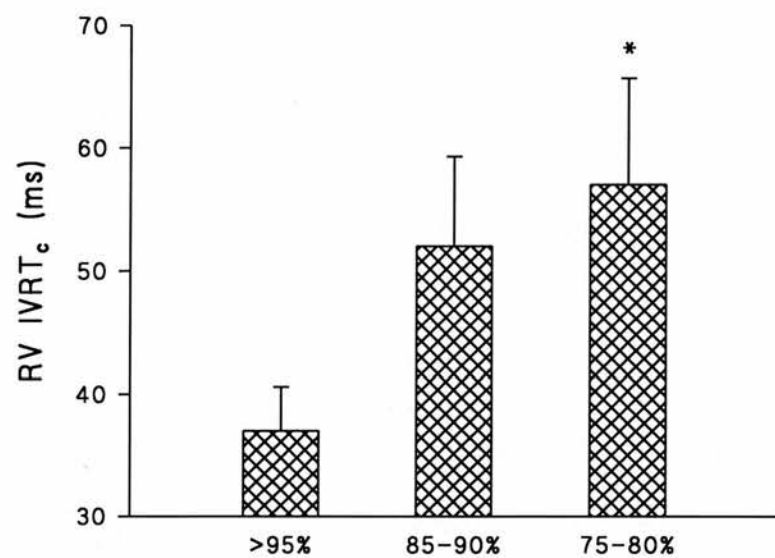
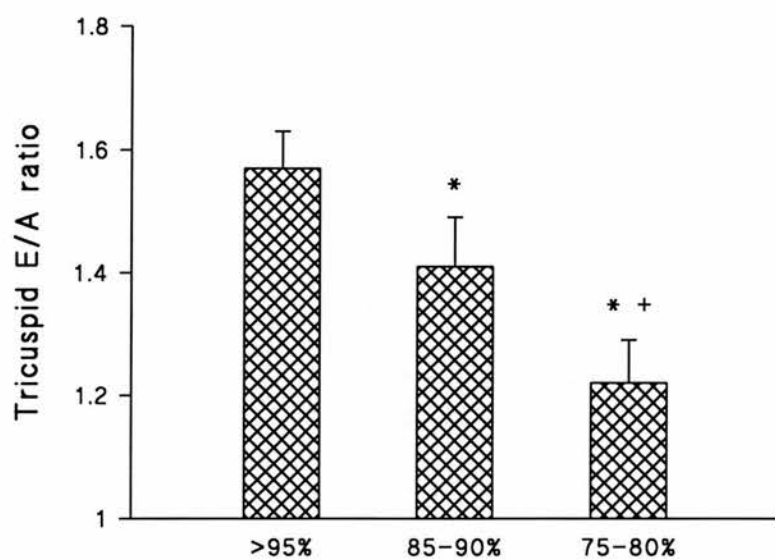
FIGURE LEGEND

Figure 4.2a, b and c.

Parameters of right ventricular diastolic function showing a) E/A velocity ratio, b) heart rate corrected isovolumic relaxation time (IVRTc) and c) heart rate corrected E-wave deceleration time (EDTc). Values are means and SEM.

* denotes significant difference from baseline ($\text{SaO}_2 > 95\%$)

⁺ denotes significant difference from SaO_2 85-90%



4.5 DISCUSSION

The present study has demonstrated abnormalities of left and right ventricular filling during hypoxaemia in normal humans. Whilst the pattern of changes in E/A ratios might conceivably be explained solely by changes in HR, the prolongation of IVRT and EDT on both sides of the heart indicate abnormal diastolic function, particularly evident when these parameters are corrected for heart rate. These haemodynamic changes are in agreement with previous work showing that chronotropic rather than inotropic effects of hypoxaemia are responsible for the increment in CO (Phillips BA et al, 1988).

IVRT is widely accepted as a measure of active myocardial relaxation and its prolongation indicates impaired diastolic function (Nishimura RA et al, 1989). Conversely, EDT represents a measure of myocardial compliance, and is shortened when compliance is decreased (Nishimura RA et al, 1989). However it is important to realise that impairment of relaxation has confounding effects on EDT. In this situation, active ventricular relaxation continues while passive filling takes place. This paradoxically increases apparent ventricular compliance with consequent lengthening of the EDT (Thomas JD et al, 1991). Thus the pattern of abnormality described in the present study is one of impaired myocardial relaxation.

These findings in humans are therefore confirmatory to previously described abnormalities of myocardial relaxation observed during hypoxia in laboratory animal systems. Studies using isolated ventricular papillary muscle have demonstrated marked impairment of relaxation during hypoxia (Frist WH et al, 1978, Nakamura Y et al 1986) whilst in isolated intact heart preparations, hypoxia was associated with an increase in ventricular chamber stiffness (Serizawa T et al 1980) as well as impairment of relaxation during diastole (Kihara Y et al 1989, Serizawa T et al 1980). The

mechanisms whereby hypoxaemia might produce these effects has been extensively studied and two possible theories are briefly considered.

Firstly, the effects of hypoxia on intracellular calcium transport may be important. Cellular hypoxia has been shown to increase intracellular calcium concentrations during diastole (Kihara Y et al 1989), due to decreased calcium uptake by the sarcoplasmic reticulum (Nayler WG et al, 1979) and perhaps other ion channel mediated effects (Rodman DM, 1992). Myocardial relaxation depends critically on rapid removal of free intracellular calcium ions to quickly reverse the calcium facilitated actin-myosin interaction which would otherwise sustain contraction (Nishimura RA et al, 1989). It is likely that hypoxia delays this process so allowing contractile elements within the cell to remain cross-linked into diastole and impairing relaxation.

The second possibility is a mechanical one, related to the concept of ventricular interaction across the interventricular septum (Stojnic BB et al, 1992). Hypoxaemia, by inducing pulmonary hypertension (Cutai M et al, 1990), increases right ventricular afterload which might cause dynamic changes in septal motion. This pattern has been observed in other forms of pulmonary hypertension (Jessup M et al, 1987, Tanak H et al, 1980) but has only been studied at much higher pulmonary artery pressures than we achieved. Although we did not study this phenomenon directly in the present study, it is conceivable that abnormal septal motion, which is associated with impairment of left ventricular filling (Stojnic BB et al, 1992), might explain some of the abnormalities observed.

Interestingly, the effects of pulmonary hypertension alone on right ventricular diastolic function have not been studied, although this is impaired in cor pulmonale (Marangoni S et al, 1992) where either pulmonary hypertension or hypoxaemia might play a role. In the present study, we have shown that whilst both right and left ventricular

relaxation is impaired by hypoxaemia, prolongation of IVRTc as a percentage of baseline is more marked in the right ventricle than in the left. Obviously baseline IVRTc was shorter in the right ventricle (with values in the present study similar to those previously published although recorded by a different method (Marangoni S et al, 1992)) but increases in pulmonary artery pressure would be expected to prolong systolic pulmonary artery flow. This would delay pulmonary valve closure and in fact shorten right ventricular IVRT. This effect would however need to be balanced by the possibility that increased pulmonary artery pressure would alter myocyte loading conditions in the right ventricle, which may directly impair relaxation as has been shown to occur in the left ventricle (Raff GL et al, 1981). In view of the finding that right ventricular IVRTc was prolonged proportionately more than left ventricular IVRTc, this may indicate that right ventricular relaxation is more sensitive to the adverse effects of hypoxaemia.

It would appear therefore that as is the case in vitro, hypoxaemia significantly impairs myocardial relaxation in humans and that these abnormalities are of similar magnitude to the diastolic filling abnormalities observed in some disease states. IVRTc in the right ventricle was prolonged by 20.3 ms which approaches the level of abnormality detected in patients with hypoxaemic COPD and pulmonary hypertension (Marangoni S et al, 1992). In the left ventricle, the observed lengthening of IVRTc (by 9.8 ms) during hypoxaemia is more difficult to place in a clinical context although is similar in magnitude to the adverse effects of very high dose infusions of angiotensin II (Clarkson PBM et al, 1994). The haemodynamic significance of these effects is therefore speculative at present as clinical studies are lacking. Whether treating hypoxaemic patients with oxygen would actually improve diastolic function has not been studied, but it is conceivable that this is one way in which oxygen therapy is of benefit to such patients.

CHAPTER 5

**ABNORMAL MYOCARDIAL REPOLARISATION IN
RESPONSE TO HYPOXAEMIA
AND FENOTEROL**

Thorax 1995;50:1062-1066.

5.1 SUMMARY

Prolongation of the QTc interval has been associated with cardiac dysrhythmias and sudden death. Recently QTc dispersion (inter-lead variability in QTc interval) has been proposed as being a more sensitive marker of repolarisation abnormalities and shown to be a more specific index of arrhythmia risk. Although hypoxaemia and fenoterol have previously been shown to prolong QTc interval this does not reflect regional myocardial repolarisation abnormalities.

Electrophysiological effects were measured at baseline and after 30 mins steady state hypoxaemia at an SaO₂ 75-80 % (study 1) and at baseline then 30 mins after inhaled fenoterol 2.4mg (study 2). From the ECG, lead II corrected QT interval (QTc) and overall corrected QT dispersion were measured using a computer linked digitising tablet according to standard criteria.

QTc dispersion was increased during hypoxia compared to baseline mean (SE) at 69(6) ms vs 50(5) ms and after fenoterol compared to baseline at 79(13) vs 46(4) ms respectively. There was also an increase in QTc interval and heart rate after fenoterol at 493(23) vs 420(6) ms and 98(3) vs 71(6) bpm respectively. There was an increase in heart rate during hypoxaemia compared to baseline at 78(3) vs 64(2) bpm, but no change in QTc interval.

Thus, both hypoxaemia and fenoterol cause myocardial repolarisation abnormalities in humans in terms of increased QTc dispersion, whereas only fenoterol increased QTc interval. This may be relevant in the aetiology of arrhythmias in patients with acute severe asthma where β -agonist therapy and hypoxaemia co-exist.

5.2 INTRODUCTION

The surface electrocardiogram has been investigated for its ability to identify those patients at risk of arrhythmias and as such represents a cheap, non-invasive and simple method. Traditionally the single measurement of QTc interval has been used to measure the recovery of ventricular excitability, widely recognised to be the most important factor in arrhythmogenesis.

QT prolongation has been shown to be a predictor of sudden death in alcoholic cirrhosis (Day CP et al, 1993), sudden cardiovascular mortality in apparently healthy individuals (Schouten EG et al, 1991), as well as sudden death in patients with ischaemic heart disease (Puddu PE et al, 1986). Recently however, QTc dispersion (interlead variability in QTc interval) has been suggested as a more sensitive and specific marker as it measures differences in regional repolarisation and has been shown to be increased in patients with HOCM at risk from ventricular arrhythmias (Buja G et al, 1993) and in patients with long QT intervals it distinguished between those with ventricular arrhythmias and those without (Day CP et al, 1990). Recently it has been found to be a more sensitive and specific marker of sudden death in heart failure than QTc interval alone (Barr CS et al, 1994).

Cardiac arrhythmias are a very common finding in patients with respiratory failure and COPD (Terlapur VG et al, 1982, Holford FD et al, 1973, Kleiger RE et al, 1974). It has been shown that depressed left ventricular diastolic performance is a predictive factor for ventricular arrhythmias during respiratory failure from COPD although the poor definition of the statistical model suggests that other factors contribute to the genesis of these arrhythmias (Raffaele AI et al, 1990). In this respect Stewart et al (Stewart AG et al, 1995) have recently shown that QTc prolongation is a better predictor of death in patients with COPD than hypoxaemia, hypercapnia or

spirometry. Interest has also surrounded the use of β -agonists which have inotropic, chronotropic and electrophysiological effects. In particular the epidemiological observation that fenoterol was associated with an increased risk of death in patients with severe asthma (Crane F et al, 1989, Grainger J et al, 1991) has suggested that this drug may predispose these individuals to arrhythmias. Interestingly the use of high dosed nebulised β -agonist has been associated with an increased risk of cardiac arrhythmias in patients with COPD (Higgins RM et al, 1987) and a further study revealed that normoxaemic COPD patients had a higher frequency of ventricular ectopy during treatment with high dose in comparison to low dose terbutaline although 24 hour Holter recordings did not reveal an increase in significant arrhythmias (Lipworth BJ et al, 1990).

We have therefore, for the first time looked at the effects of two separate stimuli which have been suggested as important factors in arrhythmogenesis in respiratory disease namely hypoxia and β -agonists, and their relative effects on QTc dispersion and QTc prolongation.

5.3 METHODS

Subjects

Sixteen healthy male volunteers aged 21-37 years were studied. There was no abnormality present on clinical history, clinical examination, 12 lead ECG, biochemical or haematological screening. Patients abstained from alcohol and caffeine for a 24 hr period prior to the study. Informed written consent to the study protocol, previously approved by the Tayside Committee for Medical Research Ethics was obtained.

Study design

Study 1.

Eight subjects were studied. An intravenous cannula was inserted into the left forearm for blood sampling. They then rested supine for at least 30 mins to obtain stable resting haemodynamics (T_0). They were then rendered hypoxaemic, as previously described, for 30 minutes by breathing a variable mixture of oxygen and nitrogen which rendered their arterial oxygen saturation between 75-80% (T_1). The hypoxic gas mixture was produced from separate cylinders of nitrogen and oxygen fitted with variable flow valves. A 12 lead ECG and venous blood samples for catecholamine and serum potassium assays, were taken at T_0 and T_1 .

Study 2: Eight subjects were studied. An intravenous cannula was inserted into the left forearm for blood sampling. Subjects then rested supine for at least 30 mins to obtain stable resting haemodynamics (T_0). Patients were then given fenoterol 2.4 mg from a metered dose inhaler (MDI) via a spacer device (12 x 0.2mg actuations with three inhalations per actuation). A 12 lead ECG and venous blood samples for serum potassium, were taken at T_0 and 30 minutes post inhalation of fenoterol (T_1).

MEASUREMENTS

QT interval and QT dispersion were measured as previously described and heart rate was calculated from five consecutive R to R intervals in lead II. Serum potassium and catecholamines were measured as previously described.

Data analysis

Comparison of values were made by multifactorial analysis of variance (Brown RA et al, 1990). A probability value of $p < 0.05$ (two-tailed) was considered to be statistically significant. Data are presented as means and standard error of the mean.

5.4 RESULTS

QTc interval.

Treatment with fenoterol significantly [$p<0.01$] increased QTc interval compared to baseline at 493(23) vs 420(6) ms respectively (Figure 5.1A). Hypoxaemia, however had no significant effect (Figure 5.1B).

QTc dispersion.

Fenoterol significantly [$p<0.05$] increased QTc dispersion compared to baseline at 79(13) vs 46(4) ms respectively (Figure 5.1C). Similarly hypoxaemia significantly [$p<0.05$] increased QTc dispersion compared to baseline at 69(6) vs 50(5) ms respectively (Figure 5.1D).

Heart Rate.

Both fenoterol [$p<0.01$]: 98(3) vs 71(6) ms and hypoxaemia [$p<0.05$]: 78(3) vs 64(2) ms significantly increased heart rate compared to baseline (Figure 5.2A).

Serum potassium.

Treatment with fenoterol resulted in a significant [$p<0.0001$] reduction in serum potassium compared to baseline at 2.93(0.12) vs 3.86(0.07) mmol/l respectively. Hypoxaemia however, had no significant effect (Figure 5.2B).

Catecholamines.

Hypoxaemia had no significant effect on either noradrenaline: 4.75(0.65) vs 3.97(0.36) nmol/l or adrenaline: 0.22(0.02) vs 0.16(0.02) nmol/l compared to baseline, respectively.

FIGURE LEGEND

Figure 5.1. Effects of hypoxaemia and fenoterol on QTc interval and QTc dispersion in normal man.

- A* Absolute QTc interval measured at baseline and after fenoterol. * represents a significant ($p<0.01$) difference between fenoterol treatment compared to baseline.
- B* Absolute QTc interval measured at baseline and during hypoxaemia.
- C* Absolute QTc dispersion measured at baseline and after fenoterol. * represents a significant ($p<0.05$) difference between fenoterol treatment compared to baseline.
- D* Absolute QTc dispersion measured at baseline and during hypoxaemia. * represents a significant ($p<0.05$) difference between fenoterol treatment compared to baseline.

In the above figure values for the same patient are connected and the mean and standard error of the mean are represented as clear circles and error bars, respectively.

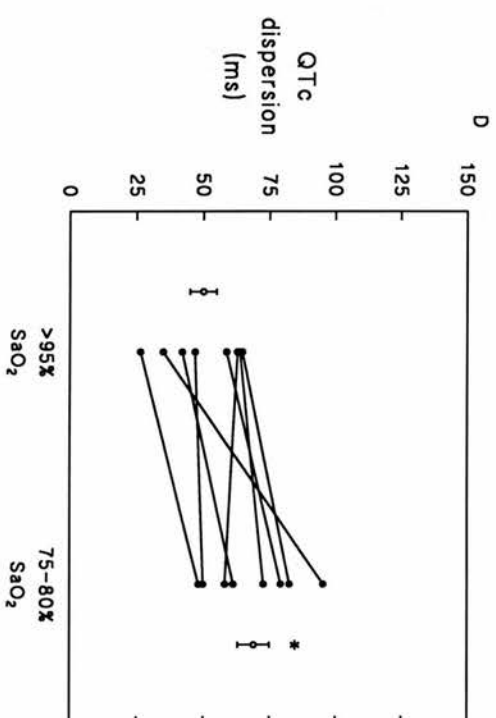
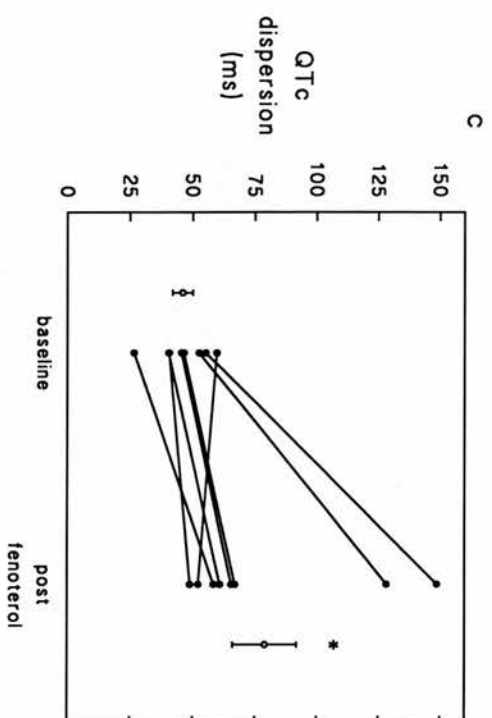
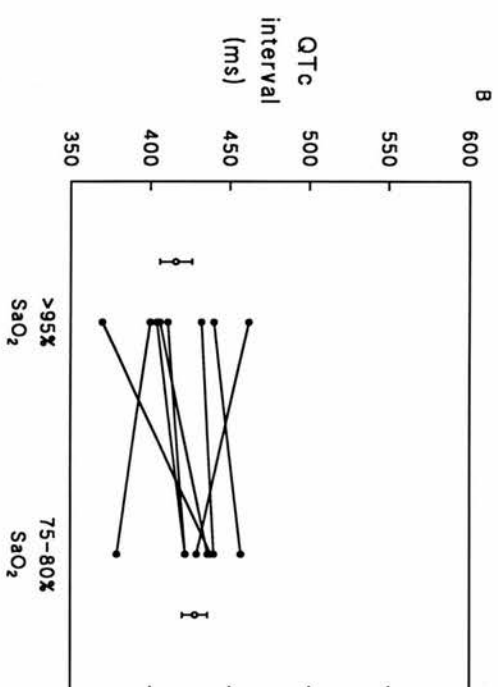
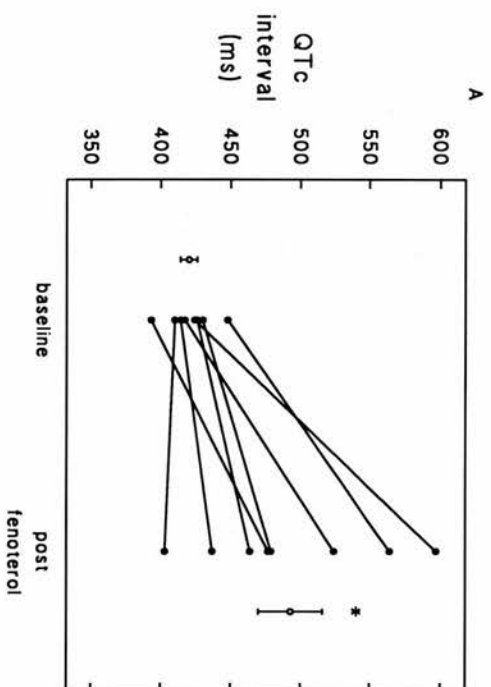
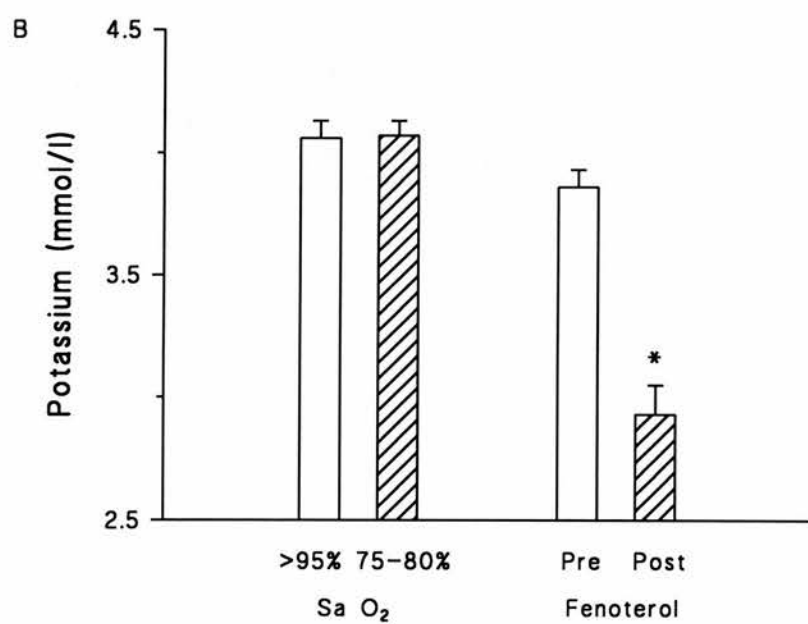
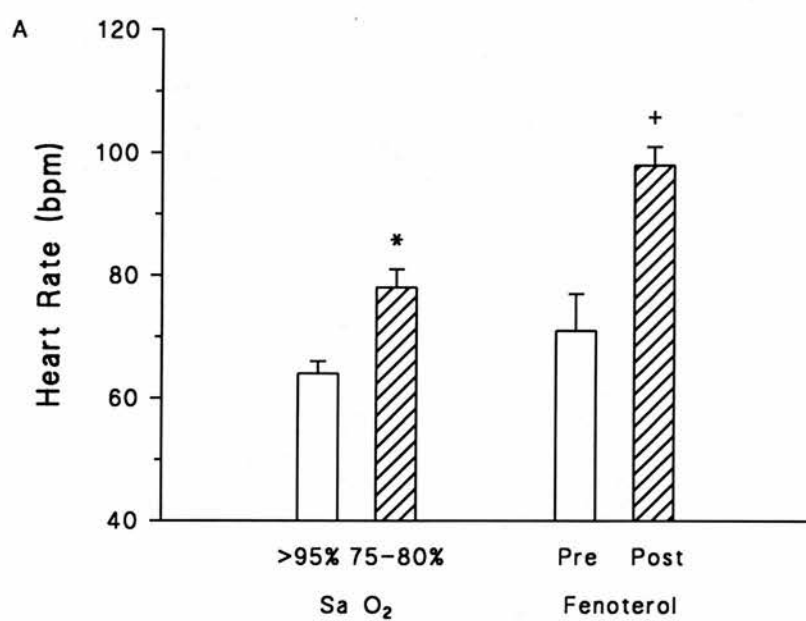


FIGURE LEGEND

Figure 5.2. Effects of hypoxaemia and fenoterol on heart rate and serum potassium in normal man.

A Left: absolute heart rate measured at baseline (clear bars) and during hypoxaemia (hatched bars). Right: absolute heart rate measured at baseline (clear bars) and after fenoterol (hatched bars). * represents a significant ($p < 0.05$) difference between hypoxaemia and baseline whilst + represents a significant ($p < 0.01$) difference between fenoterol and baseline.

B Left: serum potassium measured at baseline (clear bars) and during hypoxaemia (hatched bars). Right: serum potassium measured at baseline (clear bars) and after fenoterol (hatched bars). * represents a significant ($p < 0.0001$) difference between fenoterol and baseline.



5.5 DISCUSSION

Our results show that both hypoxaemia and fenoterol significantly increase QTc dispersion in healthy volunteers suggesting that these two stimuli cause abnormalities in myocardial repolarisation. However, only fenoterol significantly increased QTc interval and significantly decreased the serum potassium concentration compared to baseline; changes which have previously documented in normal and asthmatic subjects (Bremner P et al, 1992, Newnham DM et al, 1993).

It is thought that QTc dispersion reflects regional variation in ventricular repolarisation and existing evidence suggests that it is a powerful index of the propensity for developing life threatening arrhythmias (Buja G et al, 1993, Day CP et al, 1990, Barr CS et al, 1994). The underlying mechanism responsible for an increase in QTc dispersion is not known although it has been suggested that fibrosis may be important (Barr CS et al, 1994). However, as our results suggest dynamic physiological abnormalities must play a part as both stimuli are acute and self limiting in nature. Indeed our results are not the first to implicate dynamic variables as having an important role to play in altering QTc dispersion; Moreno et al (Moreno FL et al, 1994) have shown that QTc dispersion is decreased following successful thrombolysis after myocardial infarction demonstrating that localised ischaemia affects regional repolarisation. In the context of congestive cardiac failure no correlation was noted between increased QTc dispersion and catecholamine levels (Barr CS et al, 1994). We were unable to show any increase in circulating catecholamines during acute hypoxia although this was a small study and as such has an inherently large type 2 error. It is known that large oral doses of β -agonists do not affect catecholamine levels (Barnes PJ et al, 1982) and they were not measured in this study although Scheinin et al have shown that noradrenaline was dose dependently increased by fenoterol treatment (Scheinin M et al, 1987). It may be that hypoxia and β -agonists

have their effects on QTc dispersion by altering autonomic tone although it is likely that local metabolic and electrical disturbances may play a role. In this respect, hypokalaemia affects the electrical stability of cell membranes and is known to predispose individuals to arrhythmias and may explain, at least in part why fenoterol increases QTc dispersion.

Although hypoxia did not significantly increase QTc interval in this study, this phenomenon has previously been documented (Bremner P et al, 1992). However, it may be that QTc dispersion is a more sensitive marker of abnormal myocardial repolarisation than QTc interval alone.

With respect to our methodology there are undoubtedly limitations in all studies measuring QTc dispersion. We used a computer linked digitising tablet which has been shown by other investigators to be a reliable and accurate measure of QTc dispersion (Bhullar HK et al, 1993). Probably the most important aspect concerning methodology is the protocol to define the end of the T wave. We have thus used the most commonly used protocol (Higham PD et al, 1994) and one which has been shown to correlate with arrhythmia risk and sudden death. We also measured the QTc interval in the majority of leads in each individual although occasionally leads were omitted due to difficulty defining the end of the T-wave. We used routine ECG's to measure QTc dispersion since we felt this would have most clinical relevance and indeed no substantial evidence suggests that simultaneous ECG recording has any benefits.

What is the clinical relevance of QTc dispersion and what if any are its merits compared to our traditional measure of ventricular repolarisation, QTc interval? Firstly, much time and effort has been expended to find markers of mortality in hypoxic chronic lung disease and to find high risk patients who may benefit from further investigation or treatment. It would certainly be of interest if QTc dispersion

correlates with risk of sudden death in patients with cor pulmonale. Use of fenoterol, has been associated with an increased incidence of asthma deaths, could it be possible that this observation is due to arrhythmias occurring in the presence of abnormal myocardial repolarisation? It has been suggested that certain individuals may be more susceptible to the effects of β -agonists. In this respect it is interesting to note the large increase in QTc dispersion that occurred in two individuals in response to inhaled fenoterol (Figure 1C). Although we exceeded the standard dose of fenoterol suggested by the manufacturer, in an asthmatic attack very high doses of β -agonists may have to be taken (Windom H et al, 1990) and indeed the British Thoracic Society guidelines recommend up to 50 puffs of a β -agonist from a metered dose inhaler during a severe attack (British Thoracic Society Guidelines for management of asthma in adults, 1990). We have also shown that acute hypoxaemia increases QTc dispersion suggesting that this may have a role to play in arrhythmogenesis in acute asthma although we can infer little concerning chronic hypoxia. Compared with QTc interval, QTc dispersion, which measures ventricular repolarisation in several leads, may be a better indicator of arrhythmia risk. Indeed, it has been shown to be both a more sensitive and specific marker of sudden death than QTc interval alone (Barr CS et al, 1994). Amiodarone which is known to prolong QTc interval is used in the treatment of ventricular tachycardia. It is perhaps no coincidence that this drug also reduces QTc dispersion (Dristas A et al, 1992).

Thus, we have shown for the first time that two stimuli thought to be associated with arrhythmogenesis in respiratory disease increase QTc dispersion, namely hypoxia and β -agonists. It seems likely that this index of abnormal myocardial repolarisation will have important implications in terms of risk stratification of patients and in the development of new and the investigation of current drugs in respiratory medicine.

CHAPTER 6

**EFFECTS OF HYPERCAPNIA ON HAEMODYNAMIC,
INOTROPIC, LUSITROPIC AND ELECTROPHYSIOLOGICAL
INDICES IN NORMAL MAN**

Chest 1996;109:1215-1221.

6.1 SUMMARY

The inotropic, lusitropic and electrophysiological effects of acute hypercapnia in man are not known. Although the effects of hypercapnia on the systemic circulation have been well documented there is still some debate as to whether hypercapnia causes true pulmonary vasoconstriction *in vivo*. We have therefore evaluated the effects of acute hypercapnia on these cardiac indices as well as the interaction of hypercapnia with the systemic and pulmonary vascular beds in man.

Eight healthy male volunteers were studied using Doppler echocardiography. After resting for at least 30 mins to achieve baseline haemodynamic parameters (T_0), they were rendered hypercapnic to achieve an end-tidal CO_2 of 7 KPa for 30 mins by breathing a variable mixture of CO_2 /air (T_1). They were restudied after 30 mins recovery breathing air (T_2). Haemodynamic, diastolic and systolic flow parameters, QT dispersion (maximum-minimum QT interval measured in a 12 lead ECG) and venous blood samples for plasma renin activity (PRA), angiotensin II (ANG II) and aldosterone (ALDO) were measured at each time point.

Hypercapnia compared to placebo significantly increased mean pulmonary artery pressure 14 ± 1 vs 9 ± 1 mmHg and total pulmonary vascular resistance 171 ± 17 vs 129 ± 17 dyne.s.cm⁻⁵, respectively. Heart rate, stroke volume, cardiac output and mean arterial blood pressure were increased by hypercapnia. Indices of systolic function namely peak aortic velocity and aortic mean and peak acceleration were unaffected by hypercapnia. Similarly, hypercapnia had no effect on lusitropic indices reflected by its

lack of effect on the isovolumic relaxation time, mitral E wave deceleration time and mitral E/A wave ratio. Hypercapnia was found to significantly increase both QTc interval and QT dispersion: 428 ± 8 vs 411 ± 3 ms and 48 ± 2 vs 33 ± 4 ms respectively. There was no significant effect of hypercapnia on PRA, ANG II or ALDO.

Thus, acute hypercapnia appears to have no adverse inotropic or lusitropic effects on cardiac function although repolarisation abnormalities, reflected by an increase in QT dispersion, and its effects on pulmonary vasoconstriction may have important sequelae in man.

6.2 INTRODUCTION

Hypercapnia is a well recognised consequence of a variety of disease states. It is frequently encountered in the context of chronic obstructive airways disease and more unusually in disorders of the nervous and musculoskeletal systems. In recent years there has been much interest in the effects of hypercapnia in anaesthetic practice after the finding that mechanical ventilation may contribute to increased morbidity and mortality as a consequence of barotrauma (Hickling KG, 1990, Lachan B et al, 1982, Hamilton PP et al, 1983). This has resulted in a volume and pressure limited ventilation strategy and elevated levels of carbon dioxide, so called permissive hypercapnia (Hickling KG et al, 1990, Pesant A, 1990).

The effects of hypercapnia on the systemic circulation have been well documented (Price HL, 1960, Cullen DJ et al, 1974) although there is still some debate as to whether carbon dioxide causes true pulmonary vasoconstriction *in vivo* (Kilburn KH et al, Fishman AP et al, 1960, Rosketh R, 1966, Twining RH et al, 1968, Durand J et al, 1970, Harris P et al, 1986). Many of these studies were performed over twenty years ago and findings were sometimes based purely on changes in mean pulmonary artery pressure and where pulmonary vascular resistance was measured, it was derived from cardiac outputs calculated using the Fick principle, with errors a consequence of a changing state of respiratory gas exchange (Kierler KL, 1961, Phillips BA et al, 1988).

The advent of newer non-invasive methodology such as Doppler echocardiography has permitted a more detailed examination not only of haemodynamic effects but also of inotropic (Bennet ED et al, 1984, Cargill et al, 1995a) and lusitropic (Rokey R et al, 1985, Cargill et al, 1995b) activity. A novel marker of abnormal myocardial repolarisation, QT dispersion (Higham PD et al 1994, Kiely DG et al, 1995) has also provided us with information regarding the electrophysiological effects of different stimuli.

We have therefore evaluated for the first time the effects of acute hypercapnia on inotropic, lusitropic and repolarisation indices as well as re-examining the interaction between hypercapnia and the pulmonary circulation in the integrated physiological system of man.

6.3 METHODS

Subjects

Eight healthy male volunteers, mean age 24 years (range 21-34 years) were studied. There was no abnormality present on clinical history, examination, 12 lead ECG, echocardiography, biochemical or haematological screening. Informed written consent to the study protocol, previously approved by the Tayside Committee for Medical Research Ethics, was obtained.

Study design

Subjects attended the clinical laboratory and were studied in a supine position, rolled slightly on the left side. An intravenous cannula was inserted into the left forearm for blood sampling. Subjects then rested to obtain stable resting haemodynamics (T_0). They were then rendered hypercapnic by breathing a variable mixture of carbon dioxide and medical air to attain an end-tidal CO_2 (ET CO_2) of 7 KPa for thirty minutes (T_1) and then breathed room air for a further thirty minutes (T_2). The hypercapnic gas mixture was produced from separate cylinders of carbon dioxide and medical air fitted with variable flow valves. Measurements of pulmonary and systemic haemodynamic variables, inotropic, lusitropic and electrophysiological indices and venous blood samples for plasma renin activity (PRA), angiotensin II (ANG II) and aldosterone (ALDO) were taken at T_0 , T_1 , and T_2 .

Measurements

Systemic and pulmonary haemodynamics, systolic and diastolic filling parameters, QT interval and QT dispersion and oxygenation and end tidal carbon dioxide concentrations were measured as previously described.

Data analysis

Comparisons between serial time points on the same study day were made using multifactorial analysis of variance followed by Duncan's multiple range test. A probability value of $p < 0.05$ (two-tailed) was considered to be statistically significant. Data are presented in the text, tables and figures as means and SEM.

6.4 RESULTS

Oxygenation and ET CO₂.

Breathing the CO₂/air mixture compared to air significantly increased respiratory rate 21 ± 1 vs 13 ± 1 breaths/min, ET CO₂ 7.0 ± 0.2 vs 5.0 ± 0.3 KPa and oxygen saturation 98 ± 0.2 vs $97 \pm 0.2\%$, respectively. There was no significant difference between T₂ (thirty minutes post hypercapnia) and baseline.

Pulmonary Haemodynamics.

Hypercapnia (T₁) was associated with a significant ($p < 0.05$) increase in both MPAP and TPR compared to baseline (T₀). There was no significant difference between T₂ (thirty minutes post hypercapnia) and baseline (Figure 6.1).

Systemic Haemodynamics.

Hypercapnia (T₁) was associated with a significant ($p < 0.05$) increase in SBP, DBP, MAP, HR and CO compared to baseline (T₀). However, hypercapnia had no significant effect on SVR compared to baseline: 1102 ± 38 vs 1162 ± 78 dyne.s.cm-5. There was no significant difference between T₂ and T₀ for any of the systemic haemodynamic parameters (Figure 6.2).

Systolic flow parameters.

Hypercapnia compared to baseline had no significant effect on Av_{peak} , Acc_{peak} or Acc_{mean} . (Table 6.1)

Diastolic flow parameters.

Similarly hypercapnia compared to baseline had no significant effect on Ev_{max} , Av_{max} , E/A ratio, EDT, EDTc, IVRT or IVRTc (Table 6.1).

QT dispersion.

Hypercapnia compared to baseline had no significant effect on QT interval although QTc was significantly increased after hypercapnia. Hypercapnia also significantly increased QT dispersion compared to baseline and this was also significantly elevated after 30 minutes rebreathing air compared to baseline (Figure 6.3).

RAS activity.

Hypercapnia had no significant effect on AII, PRA or ALDO although PRA was significantly lower at T₂ compared to baseline (Table 6.2).

Table 6.1: Hypercapnia and its effects on systolic and diastolic parameters in normal man.

	T0	T1	T2
Av_{peak} (ms⁻¹)	1.20±0.08	1.26±0.10	1.15±0.05
Acc_{mean} (ms⁻²)	11.9±1.4	10.8±1.2	10.8±1.1
Acc_{peak} (ms⁻²)	26.8±4.0	24.8±3.6	28.1±3.6
Ev_{max} (ms⁻¹)	77±5	75±6	71±5
Av_{max} (ms⁻¹)	42±2	41±3	41±3
E/A ratio	1.86±0.17	1.90±0.21	1.81±0.19
EDT (ms)	121±5	123±7	114±9
EDTc (ms)	121±9	137±9	124±5
IVRT (ms)	66±5	65±4	68±3
IVRTc (ms)	74±5	70±3	72±4

There were no significant differences between T₀ (baseline), T₁ (ET CO₂ =7 KPa) and T₂ (after rebreathing air for thirty minutes) for each of the above variables.

Table 6.2. Hypercapnia and its effects on the RAS in normal man.

	T0	T1	T2
PRA (pmol/h/ml)	1.21±0.31	1.00±0.21	0.57±0.15 *
ANG II (pmol/l)	15.8±2	19.0±4.7	13.2±1.4
ALDO (pmol/l)	86.2±14.7	74.49±11.6	72.3±16.3

* represents a significant difference in PRA at T₂ (thirty minutes after rebreathing air) compared to T₀ (baseline). There were no significant differences between T₁ (ET CO₂=7 KPa) and the other time points for any of the above variables.

FIGURE LEGEND

Figure 6.1. Effects of hypercapnia on pulmonary haemodynamics and electrophysiological parameters in normal man.

Top left : absolute mean pulmonary artery pressure (MPAP) measured during normoxaemia (baseline), after 30 mins hypercapnia and 30 mins after rebreathing air, respectively.

Bottom left: absolute total pulmonary vascular resistance (TPR) measured during normoxaemia (baseline), after 30 mins hypercapnia and 30 mins after rebreathing air, respectively.

Top right: absolute QTc interval measured during normoxaemia (baseline), after 30 mins hypercapnia and 30 mins after rebreathing air, respectively.

Bottom right: absolute QT dispersion measured during normoxaemia (baseline), after 30 mins hypercapnia and 30 mins after rebreathing air, respectively.

* represents a significant ($p < 0.05$) difference between baseline and hypercapnia or between baseline and after 30 mins rebreathing air.

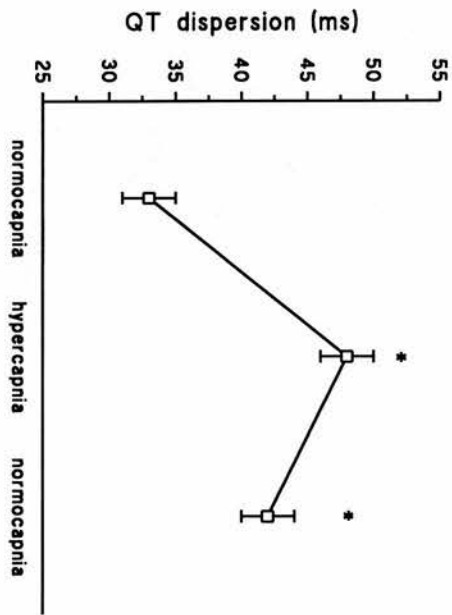
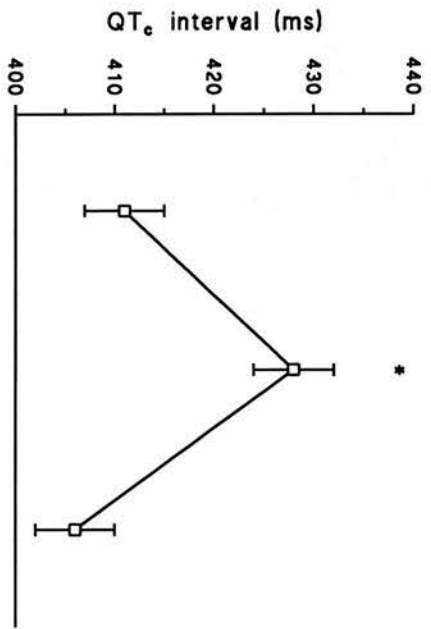
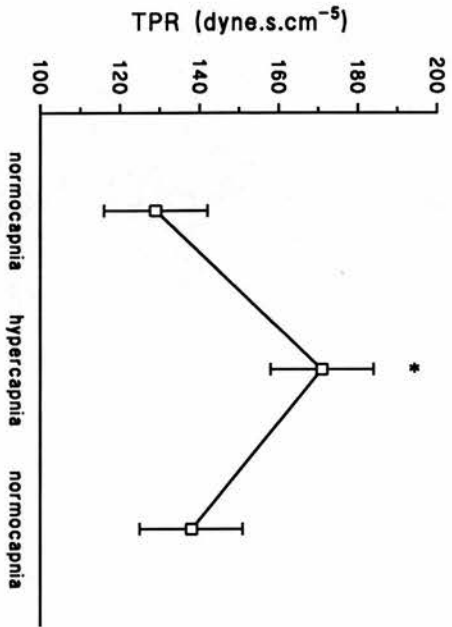
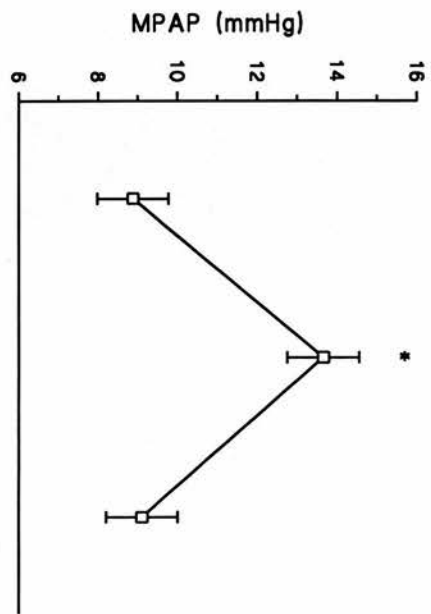


FIGURE LEGEND

Figure 6.2. Effects of hypercapnia on systemic haemodynamic parameters.

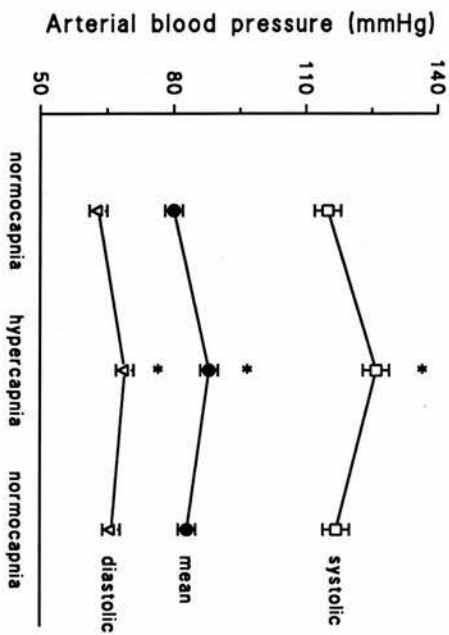
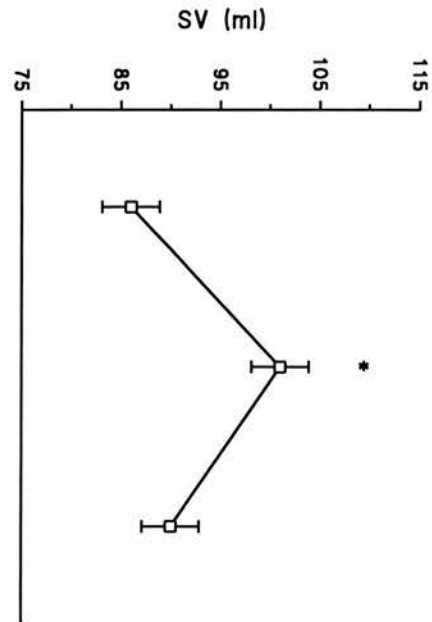
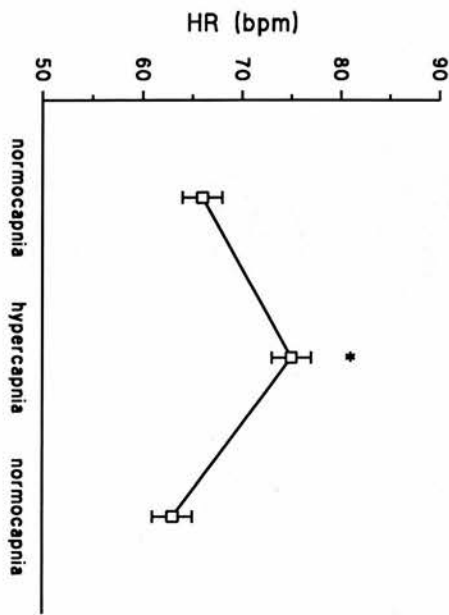
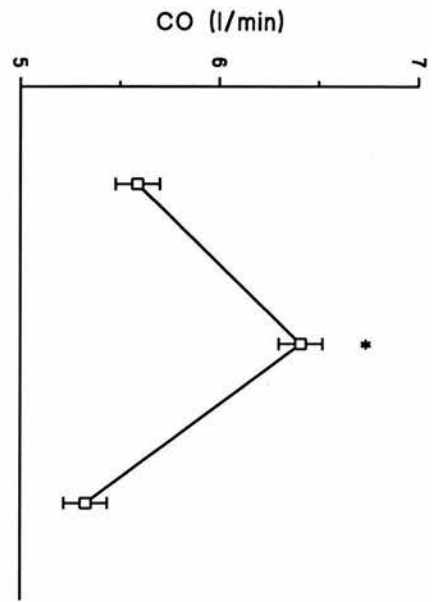
Top Left: absolute cardiac output (CO) measured during normoxaemia (baseline), after 30 mins hypercapnia and 30 mins after rebreathing air, respectively.

Bottom Left: absolute heart rate (HR) measured during normoxaemia (baseline), after 30 mins hypercapnia and 30 mins after rebreathing air, respectively.

Top Right: absolute stroke volume (SV) measured during normoxaemia (baseline), after 30 mins hypercapnia and 30 mins after rebreathing air, respectively.

Bottom Right: absolute systolic blood pressure (clear squares), mean arterial blood pressure (bold circles) and diastolic blood pressure (clear circles) measured during normoxaemia (baseline), after 30 mins hypercapnia and 30 mins after rebreathing air, respectively.

* represents a significant ($p < 0.05$) difference between baseline and hypercapnia.



6.5 DISCUSSION

We have shown that acute hypercapnia causes true pulmonary vasoconstriction *in vivo* in normal volunteers as reflected by a significant increase in both MPAP and PVR. Although acute hypercapnia had no significant inotropic or lusitropic effects it significantly increased QT dispersion suggesting that hypercapnia may cause abnormalities in myocardial repolarisation.

The effect of carbon dioxide on the pulmonary circulation in man remains controversial although the evidence appears to suggest a vasoconstrictor effect (Kilburn KH et al, Fishman AP et al, 1960, Rosketh R, 1966, Twining RH et al, 1968, Durand J et al, 1970, Harris P et al, 1986). We aimed to achieve ET CO₂ similar to that encountered in patients with exacerbations of COPD and also that found in permissive hypercapnia. Our mean ET CO₂ of 7 KPa equates with an arterial pCO₂ of approximately 7.5 KPa, and ET CO₂ is known to closely mirror the concentration of carbon dioxide in arterial blood (O'Flaherty D, 1994). Blood leaving the ventilated alveoli usually mixes with blood from both parenchymal lung tissue and with blood passing through non-ventilated alveoli, creating a venous admixture. It is this venous admixture that accounts for the normal alveolar-arterial CO₂ tension difference. The early work of Fishman (Fishman AP et al, 1960) looked at the effect of 3-5% CO₂ on the pulmonary vasculature in normal volunteers and in patients with COPD and concluded that breathing air rich in carbon dioxide had no effect on pulmonary vasoconstriction. This was in sharp contrast to work performed in animals and this apparent dichotomy was explained by Kilburn (Kilburn et al, 1969) who demonstrated pulmonary vasoconstriction in patients with COPD exposed to more severe hypercapnia. These findings have been corroborated in other studies in patients with elevated and normal mean pulmonary artery pressures (Rosketh R, 1966, Paul G et al, 1964). This study in normal man provides further support for the evidence in patient

studies that hypercapnia is a relatively weak pulmonary vasoconstrictor and that pulmonary vessels may be the exception to the rule that acidosis causes vasodilatation (Bergofsky EH et al, 1962). Thus, hypercapnia may function in man as an intrinsic mechanism diverting blood from underventilated areas of the lung in an effort to maintain ventilation perfusion matching. In contrast to previous studies we have used Doppler-echocardiography to measure haemodynamic changes in the pulmonary circulation. These non-invasive techniques have been shown to be highly reproducible (Lipworth BJ et al, 1994a) and the close correlation between Doppler PAT and MPAP as measured by right heart catheter, is well established (Dabestani A et al, 1987, Graettinger WF et al, 1987, Kitbatake A et al, 1983). We looked at two measures of pulmonary vasoconstriction; changes in MPAP and TPR. The use of total pulmonary vascular resistance does not account for any changes in the post-capillary vascular bed, as conventionally assessed by pulmonary capillary wedge pressure (PCWP). In this respect we feel it is unethical to insert Swan-Ganz catheters into normal volunteers for research purposes and the extra information this would give us is not essential. It has previously been shown that hypercapnia has no significant effects on PCWP either in patients with normal pulmonary artery pressures or those with elevated pressures occurring as a consequence of hypoxic lung disease and so effects on total pulmonary vascular resistance are reflective of changes in true PVR in pre-capillary arterioles during hypercapnia (Rosketh R, 1966). We believe therefore, that the observed changes in total pulmonary vascular resistance are a true reflection of changes in pulmonary vascular tone.

The systemic effects of hypercapnia are complex and reflect a balance between the direct effects of carbon dioxide and the secondary effects of carbon dioxide mediated via the central and autonomic nervous systems. In this study we have demonstrated significant increases in HR, SV, CO, SBP, MAP and DBP and a non-significant

reduction in SVR, changes which have previously been documented in patients with similar degrees of hypercapnia (Price HL, 1960, Cullen DJ et al, 1974).

Interestingly, although hypercapnia has been shown to be a direct myocardial depressant in the isolated heart (Price HL et al, 1955, Williams EI, 1955), we have shown no significant effects of hypercapnia on either inotropic or lusitropic indices of cardiac function measured using Doppler echocardiography. Mean and peak aortic acceleration as well as peak aortic velocity have been shown to be sensitive markers of left ventricular contractility (Bennet ED et al, 1984, Wallmeyer K et al, 1986, Bedotto JB et al, 1989, Sabbah HN et al, 1986). Although Acc_{peak} and Av_{peak} decline with increasing heart rate (Harrison MR et al, 1989) during pacing, the effects across our HR range are small and consistent with the non-significant changes observed. This suggests that the systolic contractility of the normal human myocardium is relatively resistant to the effects of acute hypercapnia. Similarly we have shown no effect on ventricular diastolic function which is an important determinant of overall cardiovascular performance and a sensitive marker of cardiac dysfunction, as reflected by the lack of effect of hypercapnia on all of the measured indices. This suggests that the secondary effects of hypercapnia on the central and autonomic nervous systems in the integrated physiological system of man are capable of antagonising the direct myocardial depressant effects of hypercapnia (Cross BA et al, 1962, Downing SE et al, 1963).

Although acute hypercapnia appears to have no significant effects on myocardial contractility, the observation that hypercapnia increases both QTc interval and QT dispersion suggests that it has significant effects on myocardial repolarisation. The finding that hypercapnia increases QT dispersion is probably of more significance since this represents differences in regional myocardial repolarisation and as such represents a putative substrate for arrhythmias. In contrast QTc interval provides no

information regarding regional repolarisation abnormalities. Evidence suggests that QT dispersion is a sensitive index of the propensity for developing life threatening arrhythmias and as such may lower the arrhythmogenic threshold in conditions where hypercapnia exists. The mechanism whereby hypercapnia causes these abnormalities in myocardial repolarisation may be related to its effects on autonomic function or elevated levels of catecholamines which have been previously demonstrated during acute hypercapnia (Sechzer PH et al, 1960). QT dispersion is a useful, easily applicable non-invasive technique. We used a computer linked digitising tablet which has been shown by other investigators to be a reliable and accurate measure of QT dispersion (Buja G et al, 1993). Probably the most important aspect concerning methodology is the protocol to define the end of the T wave. We have thus used the most commonly used protocol (Higham PD et al, 1994) and one which has been shown to correlate with arrhythmia risk and sudden death in patient studies (Barr CS et al, 1994, Farber MO et al, 1977). We used routine ECG's to measure QT dispersion since we felt this would have most clinical relevance and indeed no substantial evidence suggests that simultaneous ECG recording has any benefits.

We have also investigated the effect of acute hypercapnia on the RAS. In the absence of hypercapnia RAS activation in hypoxaemic patients is rare (Colice GL et al, 1985) suggesting a possible role for hypercapnia possibly occurring as a consequence of renal vasoconstriction or as a result of a direct cellular effect. In this study, however, we were unable to demonstrate any significant effect of hypercapnia on either PRA, ANG II or ALDO. This may be related to the brevity of our stimulus, although similar periods of hypoxia suppressed ALDO levels (Cohen EL et al, 1967). It is also possible that hypoxia and hypercapnia may need to be present in synergistic fashion to produce clinically detectable RAS activation. The significant fall in PRA thirty minutes after cessation of hypercapnia compared to baseline is consistent with the known effects of resting in the supine position, where values of PRA increase with

upright body posture and fall with time when the supine position is assumed (Tuck ML et al, 1975).

To conclude, acute hypercapnia appears to have no effects on myocardial contractility or relaxation in the integrated physiological system of man although repolarisation abnormalities reflected by an increase in QT dispersion may provide an environment for arrhythmogenesis. We have also shown that hypercapnia causes true pulmonary vasoconstriction in man. This agrees with findings in patient studies but also suggests that *in vivo* that hypercapnia has a role to play in modulating pulmonary blood flow in healthy man.

CHAPTER 7

**ANGIOTENSIN II RECEPTOR BLOCKADE AND EFFECTS ON
PULMONARY HAEMODYNAMICS AND HYPOXIC
PULMONARY VASOCONSTRICTION IN MAN**

Chest 1996;110:698-703.

(Editorial. The renin angiotensin system and the “lesser circulation”.

A role in cor pulmonale? Chest 1996;110:584-585.)

7.1 SUMMARY

We examined the hypothesis that angiotensin II (ANG II) is a modulator of pulmonary vascular tone by examining the effects of ANG II blockade on pulmonary haemodynamics during normoxaemia and hypoxaemia in normal volunteers with an activated renin angiotensin system (RAS).

Eight normal volunteers, pre-treated with furosemide, were studied on two separate occasions and received either an infusion of saralasin 5 µg/kg/min or placebo. After 20 mins they were rendered hypoxaemic, by breathing N₂/O₂ mixture for 20 mins to achieve SaO₂ 85-90% adjusted for a further 20 mins to achieve SaO₂ 75-80%. Doppler echocardiography was used to measure mean pulmonary artery pressure (MPAP), cardiac output and hence total pulmonary vascular resistance (TPR).

Saralasin compared to placebo resulted in a significant ($p<0.05$) reduction in MPAP during normoxaemia: 6.70 ± 1.0 vs 11.7 ± 1.3 mmHg, at SaO₂ 85-90%: 14.7 ± 1.4 vs 20.5 ± 1.0 mmHg and at SaO₂ 75-80%: 18.1 ± 1.9 vs 27.8 ± 1.9 mmHg respectively. Likewise saralasin compared to placebo resulted in a significant reduction in TPR during normoxaemia: 104 ± 14 vs 180 ± 20 dyne.s.cm⁻⁵, at SaO₂ 85-90%: 222 ± 24 vs 295 ± 21 dyne.s.cm⁻⁵ and at SaO₂ 75-80%: 238 ± 21 vs 362 ± 11 dyne.s.cm⁻⁵ respectively. The Δ MPAP response to hypoxaemia was likewise significantly ($p<0.01$) attenuated by saralasin infusion compared with placebo: mean difference 5.0 mmHg, 95% CI 1.9-8.08 and there was a trend towards attenuation of the Δ TPR response to hypoxaemia ($0.05<p<0.10$): mean difference 47 dyne.s.cm⁻⁵, 95% CI -10-105.

In addition to causing pulmonary vasodilatation in the presence of an activated renin angiotensin system, our results suggest that angiotensin II receptor blockade

attenuates acute hypoxic pulmonary vasoconstriction and that angiotensin II may play a role in modulating this response in normal man.

7.2 INTRODUCTION

In man hypoxaemia usually arises as a result of pulmonary disease or adverse environmental conditions such as altitude. The effects of hypoxaemia on the pulmonary vasculature have been extensively studied since Von Euler (Von Euler US et al, 1946) first described the phenomenon of hypoxic pulmonary vasoconstriction (HPV). Although HPV has beneficial effects the stimulus of chronic hypoxia results in an elevation of pulmonary artery pressure leading to the development of cor pulmonale (Fishman AP et al, 1976, Klinger JR et al, 1991).

These patients have activation of the RAS (Lang CC et al, 1992, Farber MO et al, 1977, Farber MO et al, 1982), with elevated levels of angiotensin II (ANG II). ANG II and hypoxia have both been shown to be potent pulmonary vasoconstrictors in man (Cargill RI et al, 1994). *In vitro* studies have shown that ANG II facilitates HPV in the rat and potentiates this response in dogs (Berkov S, 1974, Alexander JM et al, 1976). Furthermore, the use of ACE-inhibitors in chronically hypoxic rats has been shown to attenuate the development of pulmonary hypertension (Zakheim RM et al, 1975).

Studies in humans are rare, although recent data has shown that pre-treatment with the long acting ACE-inhibitor Lisinopril blunts acute HPV in normal volunteers compared to placebo (Cargill RI et al, 1996). The effect of ACE-inhibition could in theory be due to lowering levels of ANG II (a vasoconstrictor) or augmenting levels of bradykinin (a vasodilator) by suppressing the activity of kininase II which normally contributes to the degradation of kinins (Kramer HJ et al, 1990).

The purpose of this study was to determine the role of ANG II in the pulmonary circulation during both normoxaemia and hypoxaemia by competitive inhibition of ANG II with its analogue, saralasin (1-sar-5-val-8-ala-ANG II), which has been

shown to be a competitive antagonist of ANG II in the presence of an activated RAS, but has no effect on bradykinin degradation (Fagard R et al, 1980).

7.3 METHODS

Subjects

Eight healthy male volunteers, age (mean \pm SEM) 29 \pm 3 years were studied on two separate occasions. There was no abnormality present on clinical history, examination, 12 lead ECG, echocardiography, biochemical or haematological screening. No medications were permitted during and for one month prior to the study. Informed written consent to the study protocol, previously approved by the Tayside Committee for Medical Research Ethics, was obtained.

Study design

Subjects attended the laboratory at the same time of the day on two separate occasions, at least one week apart. Subjects were pre-treated with 4 daily doses of furosemide 40mg to activate the RAS such that saralasin would function as a pure antagonist of ANG II devoid of partial agonist activity. An indwelling intravenous cannula was inserted into each antecubital fossa, one for infusion of saralasin or placebo and the other for blood sampling. Subjects then rested supine for 30 mins to obtain stable resting haemodynamics (T₀). They then received either an infusion of 5% dextrose or saralasin 5 μ g/kg/min (Sigma Chemical Company, St Louis, USA). They were restudied after 20 minutes (T₁), and were then rendered hypoxaemic by breathing a variable mixture of oxygen and nitrogen sufficient to render arterial oxygen saturation between 85-90% (T₂) for twenty minutes adjusted for a further twenty minutes to achieve an arterial oxygen saturation of 75-80% (T₃). Measurements of pulmonary and systemic haemodynamic variables and venous blood samples for plasma renin activity (PRA) were taken at T₀, T₁, T₂ and T₃. Serum electrolytes were measured at T₀.

Measurements

Systemic and pulmonary haemodynamic indices and plasma sodium, potassium and plasma renin activity were measured as previously described

Data analysis

Comparison of values between study days or between serial time points on the same study day were made by multifactorial analysis of variance. A probability value of $p < 0.05$ (two-tailed) was considered to be statistically significant. Data are presented in the text, tables and figures as means and SEM, and where a difference between means is quoted, the 95% confidence interval for this difference is given.

7.4 RESULTS

Pulmonary Haemodynamics.

There was no significant difference in PAT, MPAP or TPR at baseline (T_0) between study days. Infusion of saralasin compared to the placebo resulted in a significant ($p<0.05$) reduction in MPAP during normoxaemia (T_1): mean difference 4.6 mmHg (95% CI 1.25-8.0), at an SaO_2 of 85-90% (T_2): mean difference 6.1 mmHg (95% CI 1.4-10.8) and a significant ($p<0.0005$) difference at an SaO_2 of 75-80% (T_3): mean difference 9.6 mmHg (95% CI 6.0-13.2), respectively (Figure 7.1A). Likewise saralasin infusion compared to placebo resulted in a significant ($p<0.05$) reduction in TPR at T_1 : mean difference 76 dyne.s.cm⁻⁵ (95% CI 29-123), at T_2 : mean difference 72 dyne.s.cm⁻⁵ (95% CI 8-136) and a significant ($p<0.0005$) reduction at T_3 : mean difference 123 dyne.s.cm⁻⁵ (95% CI 82-165), respectively (Figure 7.1B). Hypoxaemia caused a significant ($p<0.05$) increase in MPAP and TPR at T_2 and T_3 compared to baseline on both study days. Saralasin compared to placebo significantly reduced PAT during both normoxaemia, moderate and severe hypoxaemia (Table 7.1).

In terms of change in MPAP (Δ MPAP) from baseline (T_0) to severe hypoxaemia (T_3), the Δ MPAP response was significantly ($p<0.005$) attenuated by saralasin infusion compared with placebo: mean difference 7.3 mmHg, 95% CI 3.9-10.7. Likewise the Δ TPR response from T_0 to T_3 was significantly ($p<0.05$) attenuated by saralasin infusion compared with placebo: mean difference 89 dyne.s.cm⁻⁵, 95% CI 26-152. Because there was a significant fall in MPAP and TPR at T_1 compared with baseline (T_0) after saralasin infusion compared to placebo we also assessed the Δ MPAP and Δ TPR response from T_1 to T_3 . The Δ MPAP response was likewise significantly ($p<0.01$) attenuated by saralasin infusion compared with placebo: mean difference 5.0 mmHg, 95% CI 1.9-8.08 and there was a trend towards attenuation of

the Δ TPR response from T₁ to T₃ ($0.05 < p < 0.10$): mean difference 47 dyne.s.cm⁻⁵, 95% CI -10-105.

Systemic Haemodynamics.

Although, saralasin infusion compared to placebo did not significantly alter systemic haemodynamic parameters either at baseline or during hypoxia, saralasin infusion had significant effects on systemic haemodynamics compared with baseline measurements (Table 7.1). Compared to baseline (T₀), a significant ($p < 0.05$) increase in CO in response to hypoxaemia (T₃) was noted on both study days. A fall in SVR in response to hypoxaemia (T₃) was also noted on both study days although this reached statistical significance ($p < 0.05$) only during saralasin infusion. HR and MAP were unaffected by hypoxaemia on the placebo day although saralasin infusion resulted in a significant ($p < 0.05$) decrease in MAP at T₃ and T₁ compared to baseline (T₀).

Electrolytes.

There was no significant difference in serum sodium or potassium at baseline (T₀), between the study days when patients received placebo (P) or saralasin (S). Serum sodium: 137.4 ± 0.8 (P) vs 138.9 ± 0.8 (S) mmol/l and serum potassium: 3.97 ± 0.10 (P) vs 3.89 ± 0.08 (S) mmol/l.

RAS activity.

Saralasin infusion resulted in a significant ($p < 0.05$) increase in PRA at T₂ and T₃ compared to baseline (T₀): 5.25 ± 1.36 and 5.60 ± 1.31 vs 3.99 ± 0.55 ng/ml/hr respectively. Hypoxia alone was not associated with a significant change in PRA at

T₂ and T₃ compared to baseline (T₀): 3.48±0.65 and 3.29±0.58 vs 3.84±0.77 ng/ml/hr respectively. There was no significant difference in PRA at any of the time points between the two study days.

Table 7.1 Effects of ANG II receptor blockade on systemic haemodynamics and pulmonary acceleration time during hypoxaemia in normal man.

SaO₂		pre-infusion	infusion:		
		>95% (T₀)	>95% (T₁)	85-90% (T₂)	75-80% (T₃)
HR(bpm)	P	71.0±4.8	68.6±4.2	73.1±4.3	73.8±5.6
	S	67.2±3.8	64.1±3.5	68.5±4.5	71.8±5.1
MAP(mmHg)	P	84.8±4.5	81.9±3.5	82.4±3.8	86.5±3.1
	S	83.4±3.0	76.9±1.9 *	79.4±3.3	77.4±4.2 *
CO(l/min)	P	5.45±0.48	5.20±0.25	5.69±0.38	6.18±0.24 *
	S	5.36±0.34	5.06±0.17	5.42±0.36	6.10±0.39 *
SVR(dyne.s.cm⁻⁵)	P	1306±132	1262±72	1192±98	1128±48
	S	1332±80	1251±54	1260±69	1082±73 *
PAT(ms)	P	146±2	146±3	125±2 *	108±2 *
	S	152±2	158±2 *+	139±3 *+	130±5 *+

Absolute values (mean±SEM) of heart rate (HR), mean arterial pressure (MAP), cardiac output (CO), systemic vascular resistance (SVR) and pulmonary acceleration time (PAT) at each level of oxygen after pre-treatment with placebo (P) or saralasin (S).

* significantly ($p<0.05$) different from baseline (T₀).

+ significant difference between treatment with saralasin and placebo at that time point.

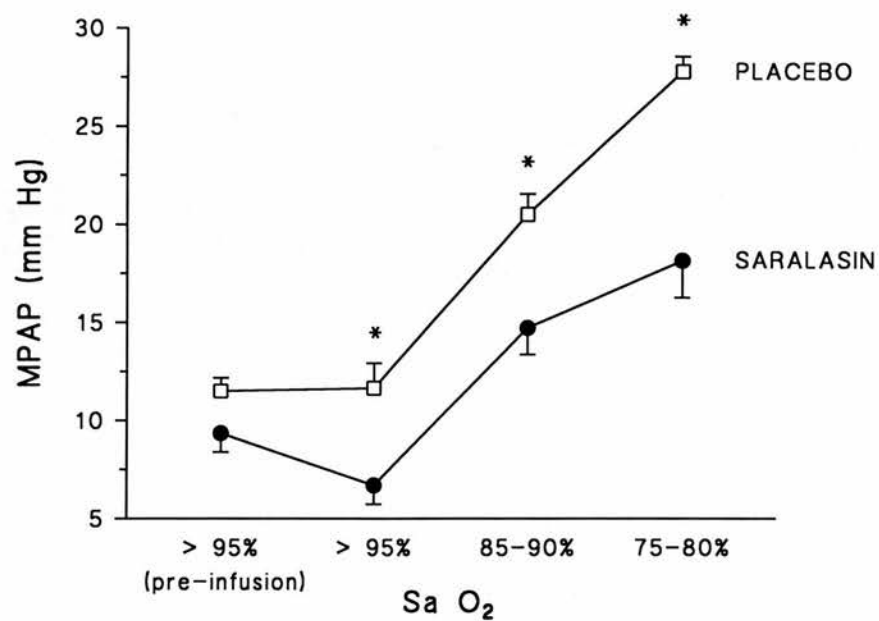
FIGURE LEGEND

Figure 7.1: Pulmonary haemodynamic response to ANG II receptor blockade during hypoxaemia in normal man.

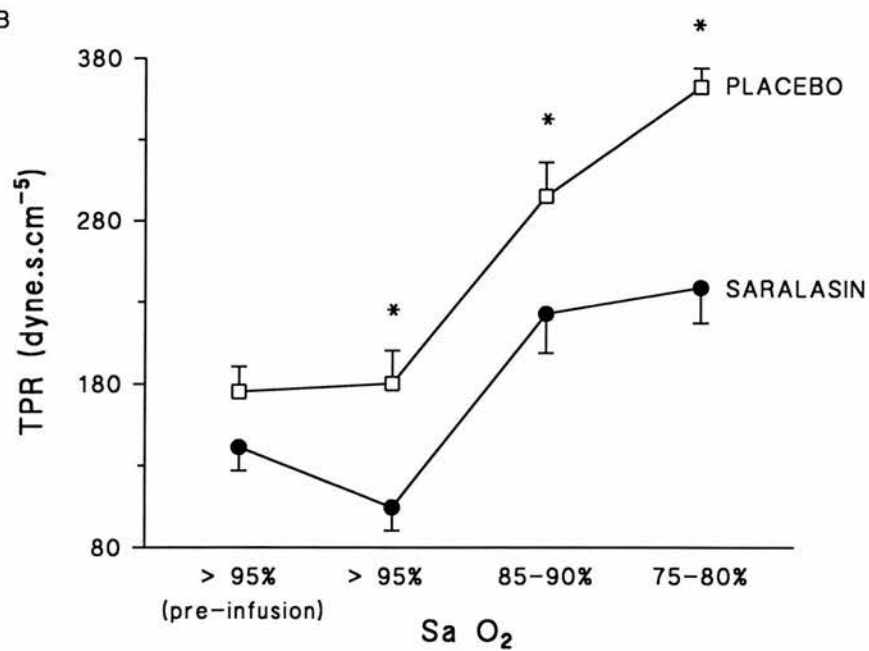
A Absolute Doppler mean pulmonary artery pressure (MPAP) measured at a) baseline before infusion with normoxaemia, b) during infusion of placebo or saralasin with normoxaemia, c) during infusion with hypoxaemia (Sa O₂ 85-90%) and d) during infusion with hypoxaemia (Sa O₂ 75-80%). * represents a significant ($p < 0.05$) difference between placebo and saralasin at each time point.

B Absolute total pulmonary vascular resistance (TPR) measured at a) baseline before infusion with normoxaemia, b) during infusion of placebo or saralasin with normoxaemia, c) during infusion with hypoxaemia (Sa O₂ 85-90%) and d) during infusion with hypoxaemia (Sa O₂ 75-80%). * represents a significant ($p < 0.05$) difference between placebo and saralasin at each time point.

A



B



7.5 DISCUSSION

Our results demonstrate that the ANG II antagonist saralasin causes pulmonary vasodilatation in the presence of an activated RAS. In this respect we have shown that absolute MPAP and TPR were significantly lower during hypoxaemia after saralasin compared to placebo and similarly that the Δ MPAP and Δ TPR responses from baseline to each level of hypoxaemia were also significantly attenuated by saralasin. There is also evidence to suggest that angiotensin II blockade may attenuate acute hypoxic pulmonary vasoconstriction reflected by a significant reduction in the Δ MPAP and a trend towards a reduction of the Δ TPR response from T₁ to severe hypoxaemia (T₃).

Saralasin is a highly soluble and stable ANG II analogue which was developed as an ANG II antagonist (Holenberg NK et al, 1976). However, saralasin also possesses partial agonist type activity. It has been demonstrated that saralasin functions as an agonist in the presence of low renin states (Case DB et al, 1976, Streeten DHP et al, 1976), whereas elevated levels of PRA and ANG II are associated with antagonism (Pettinger WA et al, 1976, Anderson GH et al, 1977). Consequently, we pre-treated our study patients with diuretics to reduce total body sodium concentration and extracellular volume to achieve elevated levels PRA. We found PRA to be the same after furosemide pre-treatment on both study days suggesting comparable degrees of RAS activation. PRA was elevated at baseline in all patients on both days, compared with our own normal reference range (0.2-2.8 ng/ml/hr), allowing saralasin to function as an ANG II antagonist rather than agonist. This antagonistic activity is supported by the findings of a significant reduction in MAP at T₁ and T₃ compared to baseline (T₀) when patients received saralasin. We also observed a significant increase in PRA at T₂ and T₃ compared to baseline (T₀), supportive of the antagonistic activity of saralasin where blockade of ANG II receptors on renin secreting cells would be

expected to result in an increase in renin secretion due to inhibition of ANG II mediated negative feedback, occurring at the level of the juxtaglomerular apparatus.

Our results agree with animal studies suggesting a possible role for ANG II in modulating HPV. Berkov suggested that ANG II was required to facilitate HPV in the saline perfused rat lung and studies in dogs suggest that ANG II infusion augments HPV (Berkov S, 1974, Alexander JM et al, 1976). Recently studies in man have shown that ANG II infusion augments HPV although not in a synergistic manner (Cargill RI et al, 1994). The finding that lisinopril pre-treatment significantly attenuated acute HPV in normal volunteers suggested that ANG II plays an important role in modulating HPV in normal man (Cargill RI et al, 1996). Although this could theoretically have been due to increased levels of bradykinin, this agent has not been shown to produce pulmonary vasodilatation in normal humans (Bonner G et al, 1990). Saralasin and lisinopril alleviate HPV to similar extents provides further evidence for a role of ANG II in modulating HPV. Although ANG II undoubtedly has important pressor effects in the pulmonary circulation studies have suggested that it is not the sole mediator of HPV. McMurty has shown that ANG II is not required for HPV *in vitro* (McMurty IF, 1984) and studies in man have shown that acute hypoxaemia does not increase ANG II levels although this may not necessarily reflect tissue angiotensin II activity in the lung (Lawrence DL et al, 1990). In this study acute hypoxaemia had no effect on PRA and ANG II blockade with saralasin only attenuated and did not abolish HPV. This supports the theory that ANG II has a role to play in modulating HPV rather than being the sole mediator of HPV.

How ANG II and hypoxia interact remains unclear although much interest has surrounded *in vitro* studies showing that pulmonary but not mesenteric arterial myocytes close potassium channels in response to hypoxia (Yuan XJ et al, 1993). This results in membrane depolarisation and inward calcium flux through voltage dependant calcium channels. ANG II is known to increase intracellular calcium

through the inositol triphosphate pathway and it has recently been suggested that it may act directly on calcium channels through its receptor (Nabika T et al, 1985, Cohen CJ et al, 1988). It may be that one subtype of potassium channel acts as a hypoxia sensor in the pulmonary vasculature and ANG II may modulate acute HPV via its effects on calcium flux or on this potassium channel via membrane voltage changes. Such that in the presence of angiotensin II blockade the hypoxic signal, with respect to pulmonary vasoconstriction, is reduced.

We have used Doppler-echocardiography to measure haemodynamic changes in the pulmonary circulation. These non-invasive techniques have been shown to be reproducible (Lipworth BJ et al, 1994) and a good correlation between Doppler PAT and MPAP as measured by right heart catheter, is well established (Dabestani A et al, 1987, Matsuda M et al, 1986, Kitbatake A et al, 1983, Graettinger WF et al, 1987). We looked at two measures of pulmonary vasoconstriction; changes in MPAP and TPR. A possible limitation of this methodology is that the use of TPR does not account for any changes in the post-capillary vascular bed, as assessed by pulmonary capillary wedge pressure (PCWP). In this respect we feel it is unethical to insert Swan-Ganz catheters into normal volunteers for research purposes. However, we believe this extra information is not essential. It is known from previous work that hypoxaemia has no significant effects on PCWP suggesting that changes in TPR are reflective of changes in true pulmonary vascular resistance in pre-capillary arterioles during hypoxia (Beard JT et al, 1991). There is no information available regarding chronic dosing with diuretics in normal volunteers, the only study available looked at the acute haemodynamic consequences of iv ethacrynic acid which showed a reduction in PCWP (Samet P et al, 1968). It would, however, be difficult to extrapolate these findings to chronic dosing and although chronic diuretic therapy may affect PCWP in normal volunteers, it is important to note that patients were exposed to both hypoxia and pre-treatment with furosemide on both study days suggesting that

these stimuli are unlikely to be responsible for the changes observed between study days. With respect to saralasin, its infusion has not been shown to affect PCWP (Mookerjee S et al, 1978). We believe therefore, that the observed changes in TPR are a true reflection of changes in pulmonary vascular tone.

We have shown for the first time in man that ANG II blockade with a specific competitive ANG II antagonist causes pulmonary vasodilatation and alleviates acute HPV in patients with an activated RAS. This suggests the possibility that ANG II is a modulator of acute HPV in normal man. Although one must be careful when extrapolating results from normal volunteer studies, the ability to cause pulmonary vasodilatation and attenuate acute HPV and therefore the stimulus for pulmonary hypertension in cor pulmonale, suggests that angiotensin II antagonists may have a role to play in chronic hypoxic lung disease either to prevent or treat the cardiopulmonary consequences of chronic hypoxaemia. The availability of orally active ANG II antagonists such as losartan may therefore provide a novel therapeutic avenue for this patient group.

CHAPTER 8

**ACUTE HYPOXIC PULMONARY VASOCONSTRICTION IN
MAN IS ATTENUATED BY TYPE 1 ANGIOTENSIN II
RECEPTOR BLOCKADE**

Cardiovascular Research 1995;30:875-880.

8.1 SUMMARY

We examined the hypothesis that angiotensin II (ANG II) is a modulator of acute hypoxic pulmonary vasoconstriction (HPV) by looking at the effect of losartan a selective type 1 ANG II receptor antagonist on acute HPV in man.

Ten normal volunteers were studied on two separate days. They either received pre-treatment with losartan 25, 50, 100, 100mg respectively on four consecutive days or placebo. They were then rendered hypoxaemic, by breathing an N₂/O₂ mixture for 20 mins to achieve an SaO₂ of 85-90% adjusted for a further 20 mins to achieve an SaO₂ of 75-80%. Pulsed wave Doppler echocardiography was used to measure mean pulmonary artery pressure (MPAP), cardiac output and hence total pulmonary vascular resistance (TPR).

Baseline MPAP and TPR (during normoxaemia) were unaffected by losartan pre-treatment compared with placebo. However, losartan significantly reduced MPAP at both levels of hypoxaemia compared to placebo: 14.7±0.7 vs 19.0±0.7 mmHg at an SaO₂ 85-90% [p<0.01] and 20.0±0.7 vs 25.7±0.8 mmHg at an SaO₂ 75-80% [p<0.05], respectively. Similarly losartan significantly reduced TPR compared to placebo: 191±9 vs 246±10 dyne.s.cm⁻⁵ at an SaO₂ 85-90% [p<0.005] and 233±12 vs 293±18 dyne.s.cm⁻⁵ at an SaO₂ 75-80% [p<0.05], respectively. Pre-treatment with losartan, however, had no significant effect on systemic vascular resistance although losartan compared to placebo resulted in a significant [p<0.05] reduction in mean arterial pressure at an SaO₂ 75-80%: 78±2 vs 87±2 mmHg.

Losartan had no effect on baseline pulmonary haemodynamics but significantly attenuated acute hypoxic pulmonary vasoconstriction, suggesting that angiotensin II plays a role in modulating this response in man via its effects on the type 1 angiotensin II receptor.

8.1 INTRODUCTION

Hypoxic pulmonary vasoconstriction (HPV) is an important homeostatic mechanism allowing blood to be diverted from areas of alveolar hypoxia. Although this undoubtedly has beneficial effects chronic hypoxia results in a continued stimulus to pulmonary vasoconstriction with consequent vascular remodelling and over time the development of cor pulmonale (Fishman et al, 1976, Magee F et al, 1988, Klinger JR et al, 1991). Interestingly patients with cor pulmonale have activation of the renin angiotensin system (RAS) (Lang CC et al, 1992, Farber MO et al, 1977, Lipworth BJ et al, 1994), with elevated levels of angiotensin II (ANG II), itself a potent direct pulmonary vasoconstrictor. Whilst in vivo studies have shown ANG II to be a pressor agent in the pulmonary circulation (Lipworth BJ et al, 1994) there is conflicting evidence as to whether ANG II is involved in a facilitatory capacity in modulating HPV (Berkov S, 1974, McMurty IF, 1984).

There has been much research into pharmacological manipulation of the hypoxic pulmonary vasoconstrictor response although concern surrounds worsening of hypoxaemia by the use of vasodilators which could theoretically have detrimental effects on ventilation perfusion matching. Interestingly the ANG II antagonist saralasin improved systemic oxygenation presumably by redistributing pulmonary blood flow and improving ventilation perfusion matching (Mookerjee S et al, 1983). ACE-inhibitors have been shown to attenuate the development of pulmonary hypertension in chronically hypoxic rats (Zakheim RM et al, 1975). A recent study in humans has shown that ACE inhibition attenuated acute HPV (Cargill RI et al, 1994). However, this cannot solely be attributed to a reduction in ANG II levels as ACE inhibitors affect many other systems in particular the kinins and prostanoids.

The purpose of this study, therefore, was to elucidate the role of ANG II and in particular the type 1 ANG II (AT₁) receptor in modulating acute HPV by competitive inhibition with losartan-potassium (DuP 753) a selective orally active specific AT₁ receptor antagonist which shows no affinity for other hormonal receptors and at a functional level, excepting ANG II, does not alter the contractile response to a variety of pressor stimuli (Timmermans PBMWM et al, 1993). Although Jaiswal et al (Jaiswal et al, 1991) reported that in vitro losartan stimulates prostacyclin release this finding has not been reproduced by other workers (Leung KH et al, 1991, Trachte GJ et al, 1990).

8.2 METHODS

Subjects

Ten healthy male volunteers, age (mean \pm SEM) 26 \pm 5 years were studied on two separate occasions. There was no abnormality present on clinical history, examination, 12 lead ECG, echocardiography, biochemical or haematological screening. Informed written consent to the study protocol, previously approved by the Tayside Committee for Medical Research Ethics, was obtained.

Study design

Subjects attended the laboratory at the same time of the day on two separate occasions, at least one week apart. Subjects were pre-treated with 4 daily doses of losartan (25mg on day 1, 50mg on day 2, 100mg on day 3,4) or placebo. They attended the laboratory 3 hours after receiving their final dose such that the drug would be studied at maximal effect. An intravenous cannula was inserted into the left forearm for blood sampling. Subjects then rested supine for at least 30 mins to obtain stable resting haemodynamics (T₀). They were then rendered hypoxaemic by breathing a variable mixture of oxygen and nitrogen which rendered arterial oxygen saturation between 85-90% (T₁) for twenty minutes then adjusted for a further twenty minutes to achieve an arterial oxygen saturation of 75-80% (T₂). Measurements of pulmonary and systemic haemodynamic variables, and venous blood samples for plasma renin activity (PRA) and electrolyte assays, were taken at T₀, T₁, and T₂

Measurements

Systemic and pulmonary haemodynamic indices and plasma sodium, potassium and plasma renin activity were measured as previously described. The intra-assay coefficient of variation for analysis of PRA was 7.6%.

Data analysis

Comparison of values between study days was made by multifactorial analysis of variance. Comparisons between serial time points on the same study day were made using Duncan's multiple range test. A probability value of $p < 0.05$ (two-tailed) was considered to be statistically significant. Data are presented in the text, tables and figures as means and standard error of the mean.

RESULTS

Pulmonary Haemodynamics.

There was no significant difference in absolute values of MPAP or TPR at baseline during normoxaemia (T₀). Losartan compared to placebo resulted in a significant [$p<0.01$] reduction in MPAP at an SaO₂ of 85-90% (T₁): mean difference 4.3 mmHg (95% CI 1.6-7.0) and a significant ($p<0.05$) difference at an SaO₂ of 75-80% (T₂): mean difference 5.6 mmHg (95% CI 2.8-8.5), respectively (Figure 8.1A). Likewise losartan compared to placebo resulted in a significant ($p<0.005$) reduction in TPR at T₁: mean difference 55 dyne.s.cm⁻⁵ (95% CI 24-86) and a significant ($p<0.05$) reduction at T₂: mean difference 60 dyne.s.cm⁻⁵ (95% CI 9-110), respectively (Figure 8.1B). Hypoxaemia was associated with a significant ($p<0.001$) increase in MPAP and TPR at T₁ and T₂ compared to baseline (T₀) on both study days.

In terms of change in MPAP (Δ MPAP) from T₀ to T₁ and T₀ to T₂, the Δ MPAP response induced by hypoxaemia was significantly ($p<0.01$) attenuated by losartan compared with placebo (Figure 8.2A): mean difference 5.2 mmHg (95% CI 2.0-8.4) and 6.6 mmHg (95%CI 3.3-9.9) respectively. Likewise the Δ TPR response induced by hypoxaemia from T₀ to T₁ and T₀ to T₂ was significantly ($p<0.01$) attenuated by losartan compared with placebo (Figure 8.2B): mean difference 70 dyne.s.cm⁻⁵ (95% CI 26-152) and 78 dyne.s.cm⁻⁵ (95% CI 29-127).

Systemic Haemodynamics.

There was no significant difference between HR, CO and SVR at each time point between the two study days although severe hypoxaemia (at T₂) resulted in a significant ($p<0.01$) increase in CO and HR and a significant decrease in SVR on both

study days. There was no significant difference between study days with respect to MAP at baseline (T_0) or T_1 , although in the presence of severe hypoxaemia (T_2), MAP was significantly ($p<0.05$) lower following pre-treatment with losartan compared with placebo: mean difference 9 mmHg (95%CI 2-16). Hypoxaemia had no significant effects on MAP on either study day (Table 8.1).

Electrolytes.

There was no significant difference in serum sodium or potassium following treatment with losartan or placebo at any time point. Similarly, hypoxia had no significant effects on serum sodium or potassium in comparison with baseline (T_0) on either study day (Table 8.2).

RAS activity.

Pre-treatment with losartan resulted in a significant ($p<0.01$) increase in PRA compared to placebo at baseline (T_0) at an SaO_2 of 85-90% (T_1) and at an SaO_2 of 75-80% (T_2). Hypoxia did not significantly affect PRA on either study day in comparison with baseline (Table 8.2).

Table 8.1. Systemic Haemodynamic effects of type 1 ANG II receptor blockade during hypoxaemia in normal man.

		SaO ₂ >95%	SaO ₂ 85-90%	SaO ₂ 75-80%
		(T ₀)	(T ₁)	(T ₂)
HR (bpm)	P:	58±2	64±2	70±3*
	L:	61±2	67±3	73±3*
MAP (mmHg)	P:	85±3	85±2	87±2
	L:	80±2	80±2	78±2+
CO (l/min)	P:	5.52±0.17	6.17±0.11	7.18±0.37*
	L:	5.39±0.24	6.22±0.33	6.96±0.36*
SVR (dyne.s.cm⁻⁵)	P:	1232±42	1098±33	994±65*
	L:	1209±39	1060±62	909±43*

* significantly (p<0.05) different from SaO₂ >95%.

+ significantly (p<0.05) different from placebo at the same time point

Values are shown as means ± standard error of the mean after prior treatment with losartan or placebo at each level of oxygenation.

HR = heart rate; MAP = mean arterial pressure; CO = cardiac output; SVR = systemic vascular resistance.

P = placebo; L = losartan.

Table 8.2. Effects of type 1 ANG II receptor blockade on serum electrolytes and PRA in normal man during hypoxaemia.

		SaO ₂ >95%	SaO ₂ 85-90%	SaO ₂ 75-80%
		(T ₀)	(T ₁)	(T ₂)
Na⁺ (mmol/l)	P:	139.1±0.4	139.3±0.	139.3±0.4
	L:	139.2±0.4	139.7±0.3	139.3±0.3
K⁺ (mmol/l)	P:	4.08±0.09	4.09±0.12	4.01±0.10
	L:	4.15±0.06	4.11±0.06	4.05±0.07
PRA (pmol/l/hr)	P	1.00±0.14	1.08±0.19	0.84±0.12
	L	7.48±1.42+	6.62±1.18+	6.46±1.02+

+ significantly ($p<0.01$) different from placebo at the same time point

Values are shown as means ± standard error of the mean after prior treatment with losartan or placebo at each level of oxygenation.

Na⁺ = serum sodium; K⁺ = serum potassium; PRA = plasma renin activity.

P = placebo; L = losartan

FIGURE LEGEND

Figure 8.1. Effects of type 1 ANG II receptor blockade on MPAP and TPR during hypoxaemia in normal man.

A Absolute Doppler mean pulmonary artery pressure (MPAP) measured at a) baseline during normoxaemia, b) at an SaO_2 85-90% and c) at an SaO_2 75-80%. Asterix represents a significant ($p<0.05$) difference between placebo and losartan at each time point.

B Absolute total pulmonary vascular resistance (TPR) measured at a) baseline during normoxaemia b) at an SaO_2 85-90% and c) at an SaO_2 75-80%). Asterix represents a significant ($p<0.05$) difference between placebo and losartan at each time point.

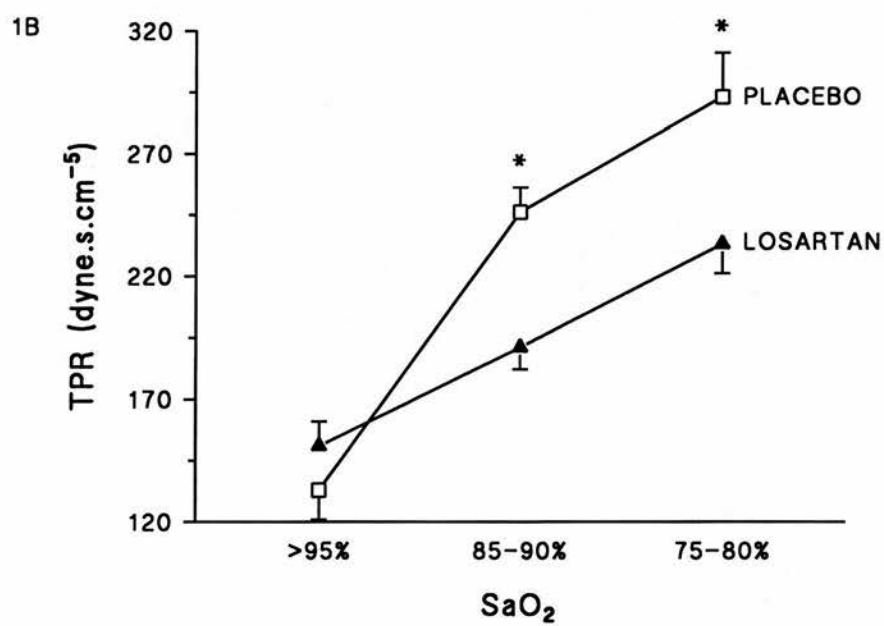
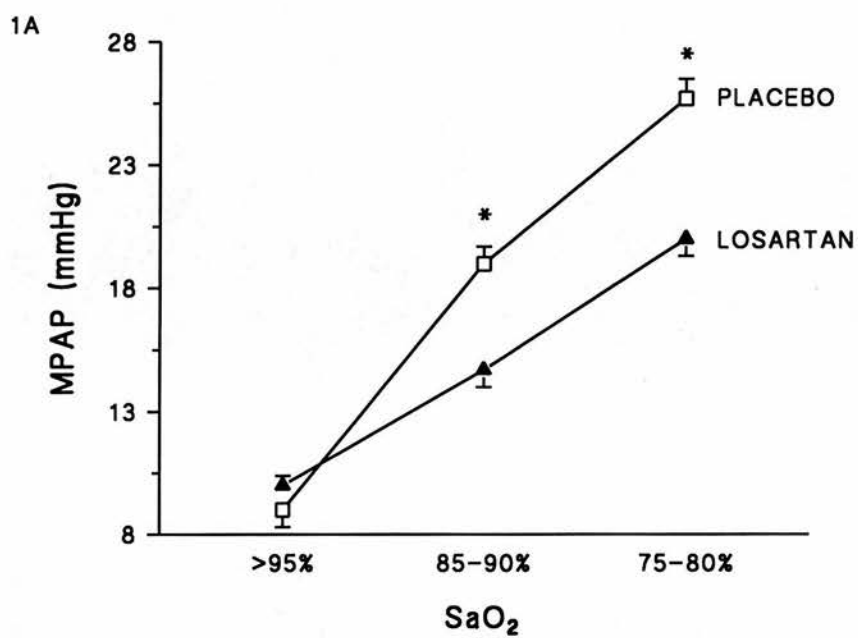


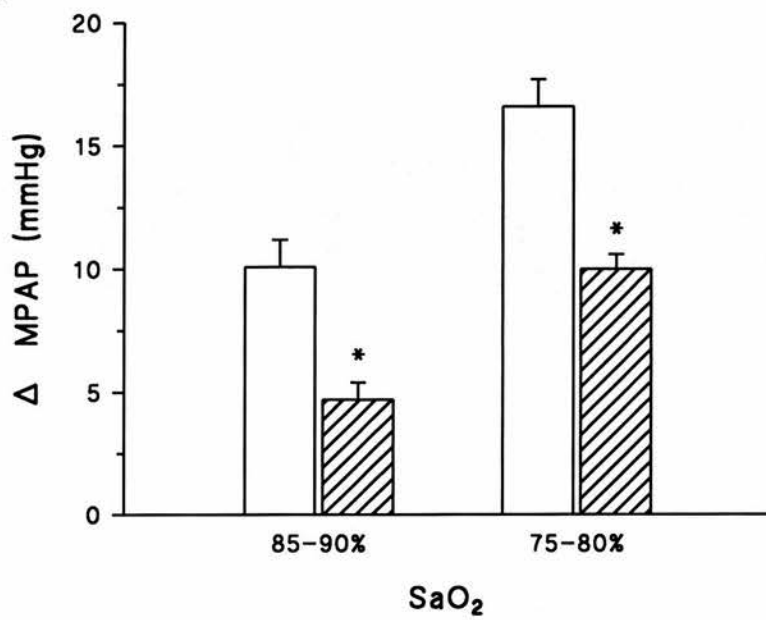
FIGURE LEGEND

Figure 8.2. Effects of type 1 ANG II receptor blockade on Δ MPAP and Δ TPR during hypoxaemia in normal man.

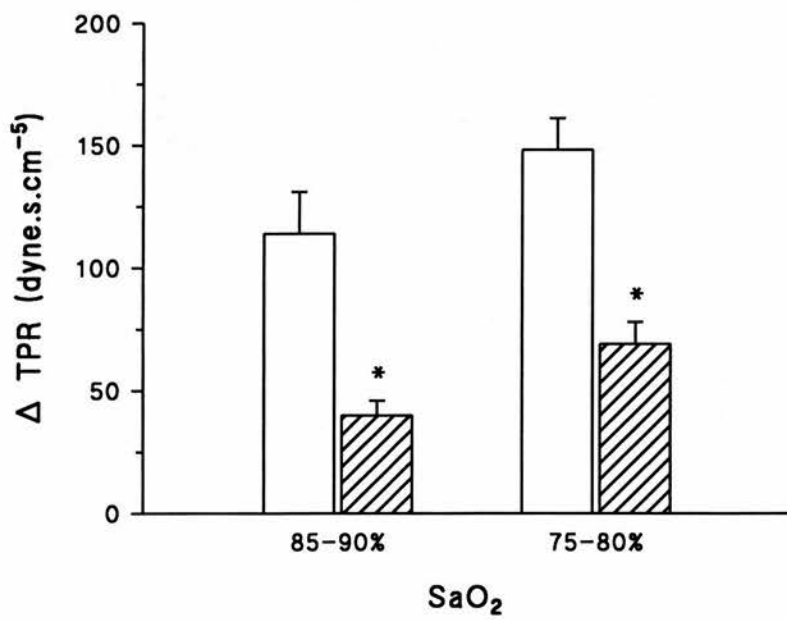
A Change in mean pulmonary artery pressure (Δ MPAP) from baseline induced by hypoxaemia a) at an SaO_2 of 85-90% (ie T_0 to T_1) and b) at an SaO_2 of 75-80% (ie T_1 to T_2). The Δ MPAP represents the mean of the individual changes. Asterix denotes that the hypoxic pulmonary vasoconstrictor response was significantly ($p < 0.01$) attenuated by losartan (hatched bars) compared with placebo (clear bars) at an SaO_2 of 85-90% and at an SaO_2 of 75-80%.

B Change in total pulmonary vascular resistance (Δ TPR) induced by hypoxaemia a) at an SaO_2 of 85-90% and b) at an SaO_2 of 75-80%, compared to baseline. The Δ PVR represents the mean of the individual changes. Asterix denotes that the hypoxic pulmonary vasoconstrictor response in man was significantly ($p < 0.01$) attenuated by losartan (hatched bars) compared with placebo (clear bars) at an SaO_2 of 85-90% and at an SaO_2 of 75-80%.

2A



2B



8.5 DISCUSSION

We have demonstrated that the selective type 1 ANG II blocker losartan attenuates acute hypoxic pulmonary vasoconstriction in man. In this respect both absolute MPAP and absolute PVR were significantly lower during hypoxia in those pre-treated with losartan compared to placebo. In addition, to correct for any possible confounding baseline effects, we have also shown that the delta-MPAP and delta-PVR responses to each level of hypoxia were significantly attenuated by losartan. This supports the hypothesis that ANG II may modulate the acute HPV response in man via its effects on the AT1 receptor.

Losartan is a potent, orally active drug which selectively blocks the AT1 receptor (Timmermans PBMWM et al, 1993) and as such provides us with a specific tool for elucidating the role of ANG II in HPV. It has been shown to produce concentration-dependent inhibition of angiotensin II induced vasoconstriction in vitro and in vivo (Christen Y et al, 1991, Rhaleb NE et al, 1991). Studies using radioligand membrane binding and autoradiography techniques have shown heterogeneity of ANG II receptors in a variety of different tissues (Timmermans PBMWM et al, 1993), however, the relative importance of the AT1 and AT2 receptor subtypes with respect to pulmonary pressor effects has not been previously documented in man. Our results support the hypothesis that ANG II has its effect primarily via the AT1 receptor since blockade of the AT1 receptor significantly attenuated this response. We also observed a significant increase in PRA after treatment with losartan during both normoxaemia and hypoxaemia. This is consistent with blockade of ANG II receptors in the juxtaglomerular apparatus resulting in renin secretion due to inhibition of ANG II mediated negative feedback. Indeed juxtaglomerular cell hypertrophy and hyperplasia has been shown to occur as a consequence of losartan therapy in rhesus monkeys (Owen RA et al, 1994). In contrast, if the AT2 receptor was as important in

mediating the acute HPV response then one would have expected a nullification or a paradoxical increase in response after treatment with losartan compared to placebo occurring as a result of elevated ANG II levels. Although the pulmonary and systemic vascular beds may respond differently to the same stimulus these results agree with findings in the systemic circulation which have shown that none of the established cardiovascular effects of ANG II can be attributed to the AT₂ receptor (Timmermans PBMWM et al, 1993).

Our findings are consistent with those in animal studies suggesting an important role for ANG II in modulating HPV. Berkov suggested that ANG II was required to facilitate HPV in the saline perfused rat lung (Berkov S, 1974) and ANG II infusion has been shown to augment HPV in dogs (Alexander JM et al, 1976). Studies in man have shown that ANG II infusion augments HPV although not synergistically (Cargill RI et al, 1994). Interestingly the finding that pre-treatment with lisinopril attenuated acute HPV in normal volunteers suggested an important role for ANG II in modulating HPV in normal man (Christen Y et al, 1991). This could theoretically have been due to increased bradykinin levels although bradykinin infusion did not produce pulmonary vasodilatation in normal volunteers (Bonner G et al, 1990). In this study we have shown that losartan attenuates HPV to a similar degree as lisinopril further implicating ANG II as having an important role in modulating HPV. Although ANG II undoubtedly has important pressor effects in the pulmonary circulation it is unlikely to be the sole mediator of HPV. McMurty has shown that ANG II is not required to produce HPV in vitro (McMurty IF, 1984) and studies in man have shown that acute hypoxaemia does not increase ANG II levels (Lawrence DL et al, 1990). This is consistent with the present study where we have shown that acute hypoxaemia had no significant effect on PRA and that ANG II blockade with losartan only attenuated rather than abolished HPV. This suggests that rather than being the sole mediator of HPV that ANG II has an important role in modulating this response.

The mechanism whereby ANG II and hypoxaemia interact remains unclear although interestingly in vitro studies have shown that pulmonary but not mesenteric vascular smooth muscle cells react to hypoxia by closing potassium channels (Yuan XJ et al, 1992). The resultant membrane depolarisation results in an increase in intracellular calcium as a result of calcium influx through voltage dependent calcium channels and consequently smooth muscle contraction. ANG II is known to increase intracellular calcium due to increased inositol triphosphate production (Nabika T et al, 1985) and it has recently been suggested that it may act directly on calcium channels (Cohen CJ et al, 1988). It may be that one subtype of potassium channel acts as a hypoxia sensor in the pulmonary vasculature and that ANG II may modulate acute HPV via its effect on calcium flux or this potassium channel via membrane voltage changes. Such that in the absence of ANG II the hypoxic signal, with respect to pulmonary vasoconstriction, is reduced.

With respect to methodology, we have used Doppler-echocardiography to measure haemodynamic changes in the pulmonary circulation. These non-invasive techniques have been shown to be highly reproducible (Lipworth BJ, 1994) and the close correlation between Doppler PAT and MPAP as measured by right heart catheter, is well established (Dabestani A et al, 1987, Kitbake A et al, 1983, Graettinger WF et al, 1987). We looked at two measures of pulmonary vasoconstriction; changes in MPAP and TPR. A possible limitation of this methodology is that the use of TPR does not account for any changes in the post-capillary vascular bed, as conventionally assessed by pulmonary capillary wedge pressure (PCWP). In this respect we feel it is unethical to insert Swan-Ganz catheters into normal volunteers for research purposes particularly as volunteers would have to be restudied on different days. The extra information this would give us is however, not essential. Both groups were exposed to hypoxia and it is known from previous work that hypoxaemia does not affect PCWP and so effects on TPR are reflective of changes in true pulmonary vascular

resistance in pre-capillary arterioles during hypoxia (Beard JT et al, 1991). With respect to ANG II blockade, an acute dosing study looking at the haemodynamic effects of losartan has shown that it does not affect PCWP (Gottlieb SS et al, 1993). We believe therefore, that the observed changes in TPR are a true reflection of changes in pulmonary vascular tone.

Thus, we have shown for the first time in man that ANG II blockade with a type 1 ANG II antagonist attenuates acute HPV. This suggests that ANG II acts as an important modulator of acute HPV in healthy humans via its effects on the AT1 receptor. One must be cautious when extrapolating from normal volunteer studies to the clinical situation. However, the ability of losartan to attenuate HPV, the initial stimulus for pulmonary vascular remodelling, suggests that it may delay or prevent progression from chronic hypoxic lung disease to cor pulmonale. Losartan may also have a role to play in the treatment of cor pulmonale where vasoreactivity is still present. This benefit may not only result from the interaction between ANG II and hypoxia with respect to the pulmonary vascular bed, but also as a consequence of blockade of the direct pulmonary pressor effects of ANG II, particularly in patients on oxygen therapy, where ANG II would be predicted to exhibit proportionately greater pressor effects (Cargill RI et al, 1994).

CHAPTER 9

**HAEMODYNAMIC AND ENDOCRINE EFFECTS OF TYPE 1
ANGIOTENSIN II RECEPTOR BLOCKADE IN PATIENTS
WITH HYPOXAEMIC COR PULMONALE**

Cardiovascular Research 1997;33:201-208.

9.1 SUMMARY

Angiotensin II (ANG II) is known to be a potent vasoconstrictor agent in the pulmonary circulation. Furthermore type 1 ANG II receptor blockade with losartan attenuates acute hypoxic pulmonary vasoconstriction in normal subjects. The aim of this study was therefore to evaluate the haemodynamic and endocrine sequelae of type 1 ANG II receptor blockade in patients with hypoxaemic cor pulmonale.

Nine patients with chronic obstructive pulmonary disease (COPD) age 67 ± 3 years with pulmonary hypertension and normal left ventricular systolic function were studied on two separate occasions in a double blind, placebo controlled, crossover study. They were randomised to receive either 50 mg of oral losartan or matched placebo. Pulsed wave Doppler echocardiography was used to measure cardiac output (CO), mean pulmonary artery pressure (MPAP) and hence systemic vascular resistance (SVR) and total pulmonary vascular resistance (TPR). Haemodynamic measurements and venous blood samples were taken at baseline and after 2 and 4 hours.

Maximal effects were observed at 4 hours where losartan compared to placebo resulted in a significant reduction in both MPAP: 28.6 ± 2.0 vs 32.4 ± 1.5 mmHg and TPR: 428 ± 40 vs 510 ± 40 dyne.s.cm⁻⁵, respectively. Similarly losartan compared to placebo resulted in a significant reduction in MAP: 87 ± 4.5 vs 93 ± 3.2 mmHg and SVR: 1293 ± 94 vs 1462 ± 112 dyne.s.cm⁻⁵, and significantly increased CO 5.58 ± 0.43 vs 5.31 ± 0.42 L/min. In addition plasma aldosterone was significantly lower after treatment with losartan compared to placebo: 76 ± 23 vs 164 ± 43 pg/ml respectively.

Thus, selective type 1 angiotensin II receptor blockade appears to have beneficial pulmonary and endocrine effects, suggesting a possible therapeutic role in the management of hypoxaemic cor pulmonale.

9.2 INTRODUCTION

Acute hypoxic pulmonary vasoconstriction (HPV) is an important phenomenon present in a wide variety of different animal species allowing blood to be diverted from areas of alveolar hypoxia so maintaining ventilation perfusion matching. Although this undoubtedly has beneficial effects it can also have deleterious effects. In particular, in chronic hypoxic lung disease, hypoxaemia provides a sustained stimulus to pulmonary vasoconstriction resulting in an elevation of mean pulmonary artery pressure (Fishman AP et al, 1976), vascular remodelling (Magee F et al, 1988) and over time the development of cor pulmonale and consequently a poor prognosis (Report of the Medical Research Council working party, 1981, Timms RM et al, 1985).

The benefits of treating pulmonary hypertension in the context of chronic hypoxic lung disease are unknown. Oxygen therapy has been shown to reduce mean pulmonary artery pressure acutely (MacNee W et al, 1988), and reduce mortality in patients with hypoxaemic cor pulmonale (Report of the Medical Research Council working party, 1981, Timms RM et al, 1985). Whether this is primarily due to improved systemic oxygenation or a reduction in right ventricular afterload is not known. Nevertheless therapy that could either prevent or treat pulmonary hypertension in these patients could conceivably reduce both mortality and morbidity and have additional benefits to oxygen therapy.

In the context of pulmonary hypertension, the role of vasoactive peptides has been extensively investigated (Cargill RI et al, 1995). It is known that angiotensin II (ANG II) is a potent pulmonary vasoconstrictor in man (Segel N et al, 1960, Lipworth BJ et al, 1994) and that patients with hypoxaemic cor pulmonale have activation of the renin angiotensin aldosterone system (RAAS) (Farber MO et al, 1977, Farber MO et al, 1982). Indeed ANG II has been shown to promote a growth response in vascular smooth muscle cells (Berk BC et al, 1989) suggesting the possibility that in addition to acting as pressor agent, ANG II could also contribute directly to vascular remodelling. Interestingly angiotensin II converting enzyme inhibitors (ACE-inhibitors) have been shown to attenuate the development of pulmonary hypertension in chronically hypoxic rats (Zakheim RM et al, 1975), although there is conflicting evidence from in vitro studies as to whether ANG II plays a facilitatory role in modulating HPV (Berkov S, 1974, McMurty IF, 1984). In normal humans however, ACE-inhibition with lisinopril (Cargill RI et al, 1996) and type 1 ANG II receptor blockade with losartan (Kiely DG et al, 1995) have been shown to attenuate acute HPV. Subsequent studies in patients with hypoxaemic cor pulmonale using ACE-inhibitors have shown variable pulmonary and systemic haemodynamic benefit (Boschetti E et al, 1985, Takada K et al, 1986, Bertoli L et al, 1986, Zielinski J et al, 1986). Whether the beneficial haemodynamic effects are a consequence of reducing ANG II levels is unknown since ACE inhibitors also increase levels of bradykinin, a vasodilator.

We have therefore evaluated for the first time the effects of selective type 1 angiotensin II receptor blockade with losartan, in patients with hypoxaemic cor pulmonale.

9.3 METHODS

Subjects

Nine patients (6 male, 3 female), with clinically stable cor pulmonale secondary to hypoxaemic cor pulmonale (mean age \pm standard error of the mean 67 \pm 3 years), were included in the study after attending a screening visit to assess inclusion criteria and characterise the study population. All had spirometric evidence of obstructive airways disease (FEV₁/FVC<70%) and arterial hypoxaemia whilst breathing air (PaO₂<8.5 KPa) and had or gave a history of having peripheral oedema. On echocardiography subjects were required to be in sinus rhythm, have normal left ventricular function, no evidence of valvular heart disease and a resting mean pulmonary artery pressure, whilst breathing room air, of at least 25 mmHg. In addition, all subjects had evidence of reversible, dynamic pulmonary vasoconstriction as assessed by $\geq 10\%$ fall in MPAP on breathing 60% oxygen for 30 minutes. All subjects were taking inhaled bronchodilators (beta-agonist n=9; anticholinergic n=6) and inhaled steroids, five patients were taking oral loop diuretics and 6 patients used domiciliary oxygen for at least 15 hours per day. Medications were unchanged throughout the study period. The summary demographic data for this patient group are given in Table 1. All subjects gave informed written consent to the study protocol previously approved by the Tayside Committee for Medical Research Ethics and conforming with the principles outlined in the Declaration of Helsinki.

Study design

Subjects were studied at the same time of the day on two separate occasions at least one week apart in a randomised, double blind placebo controlled, cross-over design. Patients taking regular diuretics were asked to omit their morning dose of diuretic on each visit. On each study day, an intravenous cannula was sited in the right forearm for venous blood sampling. Subjects then remained semi-recumbent throughout and were studied whilst breathing room air. Patients then received either 50mg of oral losartan potassium (Merck Sharp and Dohme Ltd, Hertfordshire, UK) or placebo. Haemodynamic parameters were measured and blood samples were taken at baseline after subjects had rested for at least 30 minutes to obtain stable resting haemodynamics (T_0), 2 hours (T_1) and 4 hours (T_2) after administration of either losartan or placebo.

Measurements

Systemic and pulmonary haemodynamic parameters, indices of plasma renin activity and plasma creatinine were measured as previously described. The short term coefficient of variability for MAP was 8.2%, CO was 9.6%, SVR was 5.3%, PAT was 3.2%, MPAP was 5.8% and TPR was 12.4 %. The intra-assay coefficient of variation for analysis of PRA was 6.50% and plasma aldosterone was 8.77%.

Data analysis

Comparison of values between study days was made by multifactorial analysis of variance and where there was a significant difference the mean difference and 95% confidence intervals for the mean difference are given (95% CI). Comparisons

between serial time points on the same study day were made using Duncan's multiple range test. A probability value of $p < 0.05$ (two-tailed) was considered to be statistically significant. Data are presented as means and SEM.

9.4 RESULTS

Systemic Haemodynamics

There were no significant differences in absolute values of HR, SV, CO, MAP or SVR at baseline between study days. Although there was no significant difference in either MAP or SVR after treatment with losartan or placebo at 2 hours, both MAP ($p<0.05$): mean difference 5.7 mmHg (95% CI 0.4-10.9) and SVR ($p<0.01$): mean difference 169 dyne.s.cm⁻⁵ (95% CI 71-267) were significantly lower 4 hours after treatment with losartan compared to placebo (Figure 9.2). Although losartan compared to placebo had no significant effects on SV, CO was significantly ($p<0.05$) higher 4 hours after losartan compared to placebo: mean difference 0.27 l/min (95% CI 0.04-0.50), (Table 9.2). Figure 9.3 shows the changes in SVR 4 hours after administration of losartan compared to placebo for individual patients. Whilst there was no significant difference in HR between study days at 4 hours, HR was significantly higher 4 hours after treatment with losartan compared to baseline (Table 9.2).

Pulmonary Haemodynamics

There was no significant difference in absolute values of MPAP or TPR at baseline (T_0) between study days. Losartan compared to placebo resulted in a significant ($p<0.01$) reduction in MPAP at 2 hours (T_1): mean difference 3.1 mmHg (95% CI 1.6-4.6) and a significant ($p<0.05$) reduction at 4 hours (T_2): mean difference 3.8 mmHg (95% CI 0.3-7.3), respectively. Although there was no significant difference in TPR between treatment with losartan and placebo at 2 hours, TPR was significantly ($p<0.05$) lower 4 hours after treatment with losartan compared to placebo: mean difference 82 dyne.s.cm⁻⁵ (95% CI 15-150). In addition both MPAP and TPR were significantly ($p<0.05$) lower 4 hours after treatment with losartan compared to

baseline (Figure 9.4). Figure 8.3 shows the changes in TPR 4 hours after administration of losartan compared to baseline for individual patients.

RAAS Activity and serum creatinine

There were no significant differences in absolute values of PRA, plasma aldosterone or serum creatinine at baseline between study days. Although there were no significant differences between study days for PRA either at baseline or 2 and 4 hours after administration of losartan or placebo, PRA was significantly increased 4 hours after administration of losartan compared to baseline. There were significant ($p<0.05$) falls in plasma aldosterone with time on both study but in addition losartan compared to placebo resulted in a significant ($p<0.05$) reduction in plasma aldosterone at 4 hours (T_2): mean difference 89 pg/ml (95% CI 19-159). There were no significant differences in plasma creatinine at baseline (T_0), T_1 or T_2 between study days or with time on either study day (Table 9.3).

Oxygen saturation

There were no significant differences in oxygen saturation between days on which patients received placebo or losartan at baseline (T_0): 91.4 ± 1.2 vs 91.1 ± 1.1 %, at T_1 : 90.7 ± 1.1 vs 90.7 ± 1.1 %, or at T_2 : 90.7 ± 1.2 vs 90.8 ± 1.1 % respectively, or with time on either study day.

Table 9.1. Baseline characteristics of patients with COPD.

FEV ₁ (L)	0.78±0.09
FEV ₁ % predicted (%)	30±2
FEV ₁ /FVC	43±3
Pa O ₂ (KPa) on air	7.01±0.24
Pa CO ₂ (KPa) on air	6.84±0.28
MPAP (mmHg)	32±2

The above values are expressed as means± standard error of the mean for respiratory function and pulmonary haemodynamics in the patient group studied.

FEV₁= forced expiratory volume in one second; FEV₁/FVC = ration of FEV₁ to forced vital capacity; MPAP = mean pulmonary artery pressure.

Table 9.2. Systemic haemodynamic effects of type 1 ANG II receptor blockade in patients with cor pulmonale complicating COPD.

		T ₀	T ₁	T ₂
CO (l/min)	P	5.47±0.42	5.56±0.49	5.31±0.42
	L	5.26±0.32	5.37±0.46	5.58±0.43 *
SV (ml)	P	79±7	79±7	75±7
	L	76±6	78±6	76±6
HR (bpm)	P	73±2	73±2	75±2
	L	75±3	73±3	81±4 ⁺

Values are given as mean±standard error of the mean.

* significantly (p<0.05) different from placebo at the same time point.

⁺ significantly (p<0.05) different from T₀.

CO = cardiac output; SV = stroke volume; HR = heart rate.

P = placebo; L = losartan.

Table 9.3. Effects of type 1 ANG II receptor blockade on RAAS activity and serum creatinine levels in patients with cor pulmonale complicating COPD.

		T ₀	T ₁	T ₂
PRA (ng/ml/hr)	P	4.21±1.50	4.97±1.92	4.40±1.38
	L	4.54±1.62	5.66±1.97	9.46±2.56 ⁺
Aldosterone (pg/ml)	P	238±50	187±45 ⁺	164±43 ⁺
	L	179±43	142±45	76±23 ^{*+}
Creatinine (μmol/l)	P	106±11	102±11	101±9
	L	101±10	99±11	99±9

Values are given as mean±standard error of the mean.

* significantly (p<0.05) different from placebo at the same time point.

⁺ significantly (p<0.05) different from T₀.

PRA = plasma renin activity.

FIGURE LEGEND

Figure 9.1. Validation of Doppler-derived measures of MPAP.

Upper: Data comparing pulmonary acceleration time (PAT) and simultaneously measured mean pulmonary artery pressure (MPAP) during right heart catheterisation in patients with suspected pulmonary hypertension.

Lower: Bland and Altman plot comparing mean pulmonary artery pressure measured during right heart catheterisation ($\text{MPAP}_{\text{catheter}}$) and that estimated using pulmonary acceleration time (MPAP_{PAT}). Mean difference 0.88 mmHg, SD of the difference 2.69 mmHg.

“r” = correlation co-efficient

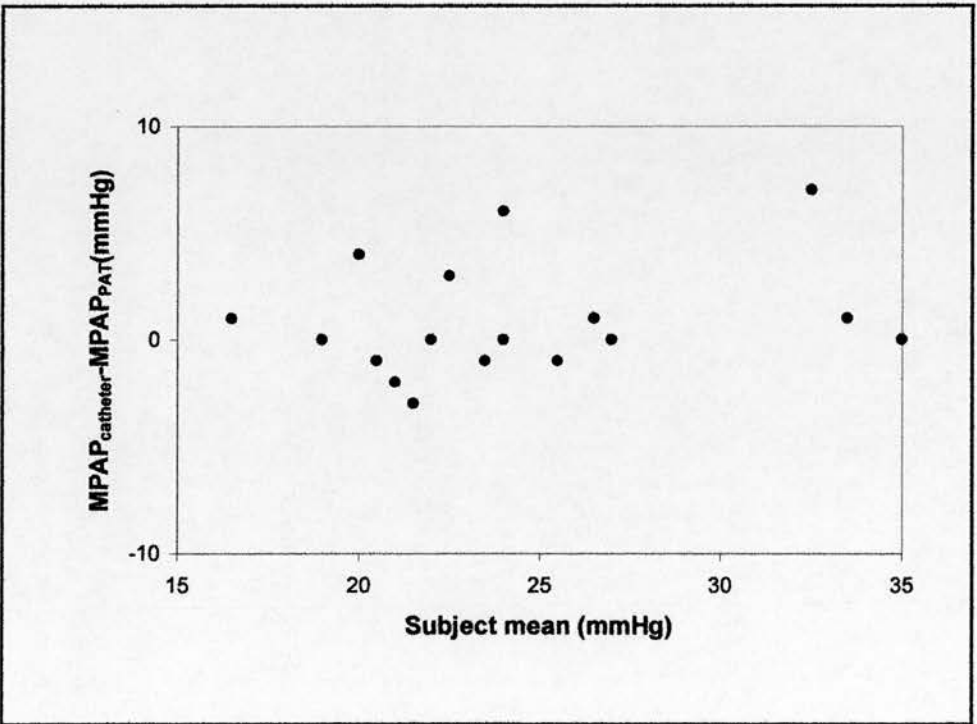
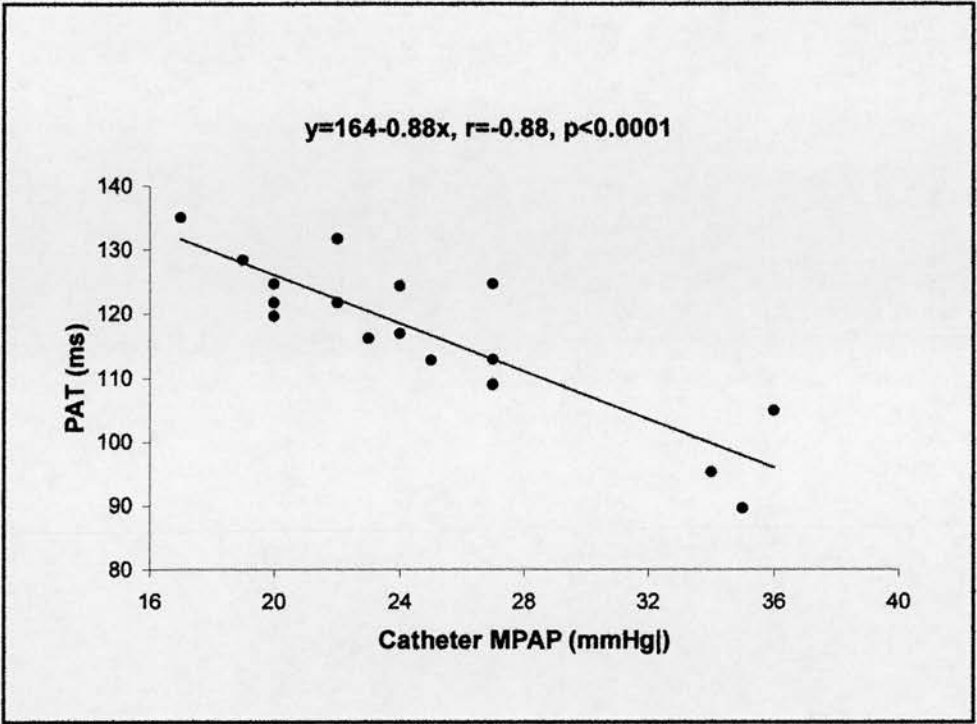


FIGURE LEGEND

Figure 9.2. Systemic haemodynamic effects of type 1 ANG II receptor blockade in patients with cor pulmonale complicating COPD.

Upper: Mean arterial blood pressure (MAP) measured at baseline, after 2 hours and after 4 hours.

Lower: Systemic vascular resistance (SVR) measured at baseline, after 2 hours and after 4 hours.

Treatment with losartan is represented by the bold triangles whereas treatment with placebo is represented by the open circles. Values are given as mean \pm standard error of the mean. * represents a significant ($p<0.05$) difference between placebo and losartan at that time point whereas a ⁺ represents a significant ($p<0.05$) difference between that time point and baseline.

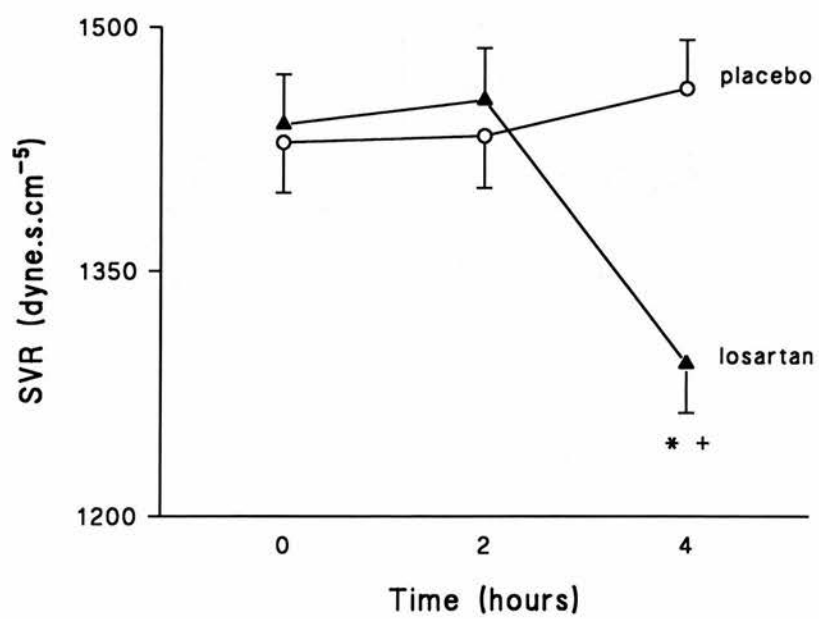
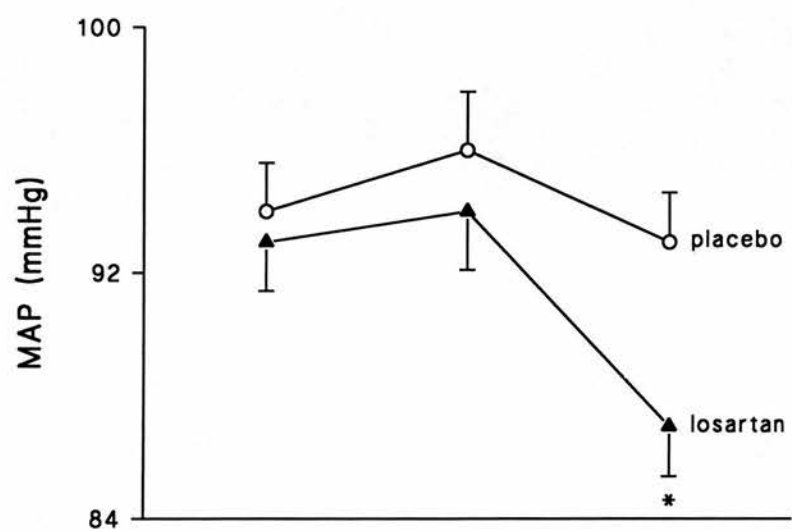


FIGURE LEGEND

Figure 9.3. Effects of type 1 ANG II receptor blockade on changes in vascular resistance in patients with cor pulmonale complicating COPD.

Upper: Total pulmonary vascular resistance (TPR), measured at baseline and 4 hours after acute administration of losartan 50 mg in 9 patients with hypoxaemic cor pulmonale.

Lower: Systemic vascular resistance (SVR), measured at baseline and 4 hours after acute administration of losartan 50 mg in 9 patients with hypoxaemic cor pulmonale.

Values are given as mean \pm standard error of the mean. * = significant ($p<0.05$) difference 4 hours after administration of losartan compared to baseline.

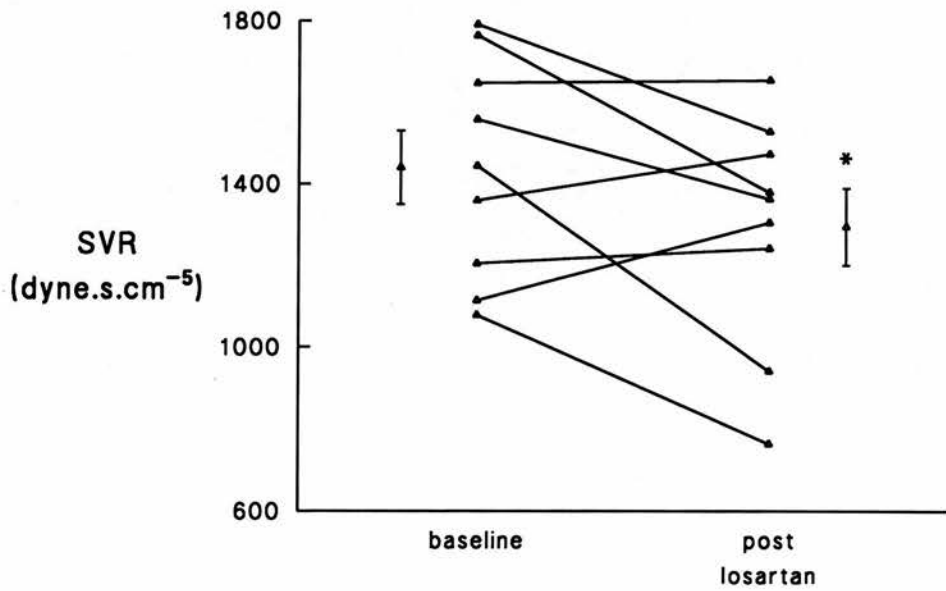
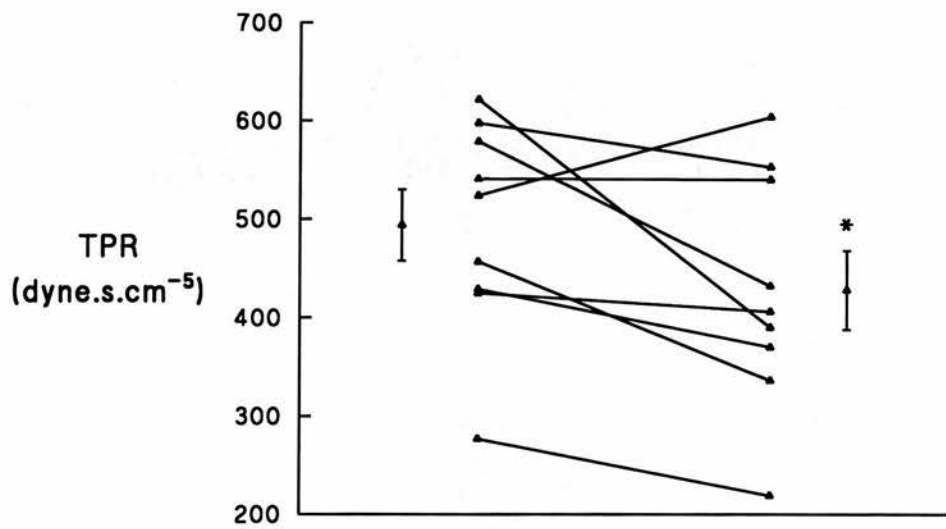


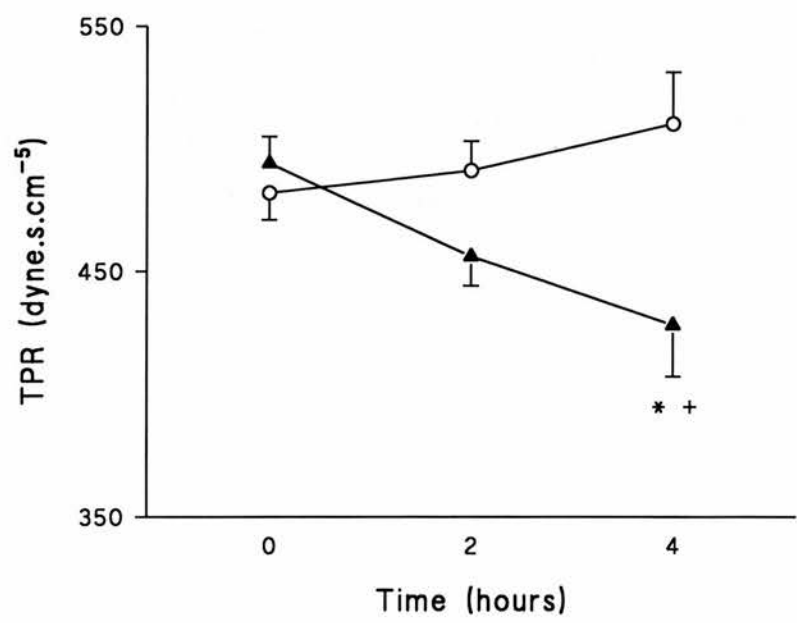
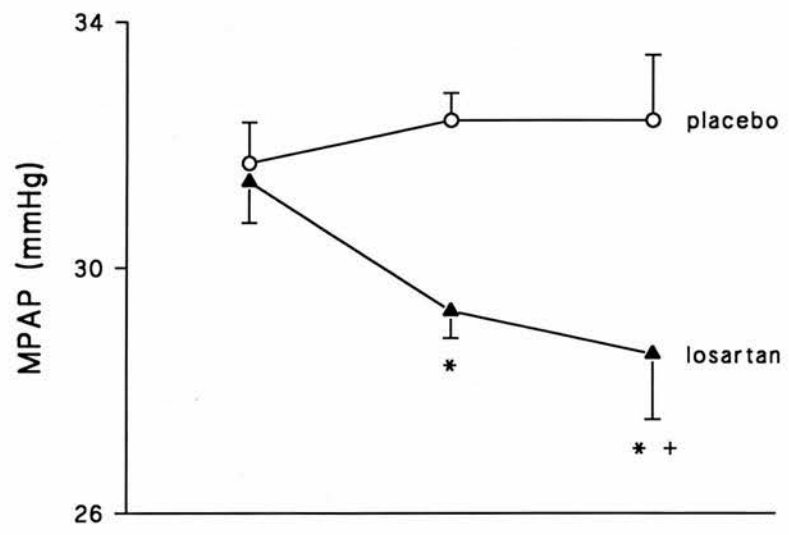
FIGURE LEGEND

Figure 9.4. Pulmonary Haemodynamic effects of type 1 ANG II receptor blockade in patients with cor pulmonale complication COPD.

Upper: Doppler mean pulmonary artery pressure (MPAP) measured at baseline, after 2 hours and after 4 hours.

Lower: Total pulmonary vascular resistance (TPR) measured at baseline, after 2 hours and after 4 hours.

Treatment with losartan is represented by the bold triangles whereas treatment with placebo is represented by the open circles. Values are given as mean \pm standard error of the mean. * represents a significant ($p<0.05$) difference between placebo and losartan at that time point whereas a ⁺ represents a significant ($p<0.05$) difference between that time point and baseline.



9.5 DISCUSSION

We have demonstrated for the first time that the selective type 1 ANG II receptor blocker losartan has beneficial pulmonary haemodynamic effects in hypoxaemic cor pulmonale without altering oxygen saturation. In this respect both MPAP and TPR were significantly lower after treatment with losartan compared to placebo, although this effect was of small magnitude and of uncertain clinical relevance. In addition type 1 ANG II receptor blockade appears to have beneficial endocrine effects reflected by significantly lower levels of plasma aldosterone after treatment with losartan compared to placebo. We also noted a significant reduction in both MAP and SVR as well as a small but significant increase in cardiac output after treatment with losartan. This suggests that angiotensin II blockade may have a therapeutic role in the management of hypoxaemic cor pulmonale.

Losartan a potent, orally active type 1 angiotensin II receptor blocker (AT₁) has provided us with a tool for elucidating the role of ANG II in the pulmonary circulation in man. It is a selective orally active selective AT₁ receptor antagonist which shows no affinity for other hormonal receptors and at a functional level excepting ANG II it does not alter contractile response to a variety of different stimuli (Timmermans PBMWM et al, 1993) and has been shown to produce concentration-dependent inhibition of angiotensin II induced vasoconstriction in vitro and in vivo (Christen Y et al, 1991, Rhaleb NE et al, 1991). Losartan undergoes an important first pass effect and is extensively transformed to its active metabolite EXP 3174 reaching peak concentrations between 2 and 4 hours after oral administration of losartan (Munafò A et al, 1992). Early studies in normal volunteers have confirmed antagonism of pressor responses to exogenous administration of angiotensin I and ANG II with

approximately 60-70 % inhibition of control responses at maximum effect (3 hours) (Christen Y et al, 1991, Christen Y et al, 1991). Although the interaction of losartan with the eicosanoid system has been observed in animals with high concentrations of the drug by some workers (Jaiswal N et al, 1991, Bertolino F et al, 1994) it seems unlikely that this accounts for the observed effects to a significant degree. Indeed conflicting results surround evidence suggesting that losartan stimulates prostacyclin synthesis (Jaiswal N et al, 1991, Leung KH et al, 1991, Trachte GJ et al, 1990).

We examined the acute haemodynamic effects of a single dose of oral losartan. Our patients had activation of the RAAS system reflected by elevated levels of PRA at baseline compared with our own normal reference range (0.2-2.8 ng/ml/hr). We also observed a significant increase in PRA 4 hours after administration of losartan compared to baseline consistent with blockade of the type 1 ANG II receptor in the juxtaglomerular apparatus resulting in renin secretion due to inhibition of ANG II mediated negative feedback.

In terms of both systemic and pulmonary haemodynamic changes we observed maximal effect at 4 hours. Whether the peak effect may have occurred after 4 hours or if larger doses of losartan would achieve greater pulmonary haemodynamic benefit or whether this would be limited by systemic haemodynamic effects is not answered by this study. Interestingly acute dosing studies in patients with congestive cardiac failure have shown no greater systemic haemodynamic effects with doses of losartan of greater than 25 mg of losartan and noted falls in MAP and SVR after 2 hours with peak effect observed between 4 and 12 hours and an effect still seen at 24 hours (Gottlieb SS et al, 1993). This study does however, suggest that angiotensin II contributes to the maintenance of both pulmonary and systemic vascular tone in patients with hypoxaemic cor pulmonale. Although ANG II has been shown to have

pressor effects in man (Segel N et al, 1960, Lipworth BJ, 1994), studies using ACE inhibitors in patients with hypoxaemic cor pulmonale have not consistently shown a pulmonary haemodynamic benefit possibly as a consequence of inclusion criteria (Boschetti E et al, 1985, Takada K et al, 1986, Bertoli L et al, 1986, Zielinski J et al, 1986). We have, however, specifically excluded patients who did not have demonstrable pulmonary vascular reactivity as assessed by the absence of a significant fall in mean pulmonary artery pressure in response to acute oxygen administration. In this respect we feel it is important that patients who are included in vasodilator trials have at least some dynamic component to their pulmonary hypertension, a situation analogous to studying bronchodilator therapy, where the degree of airway reversibility is established before evaluating the impact of therapy.

We observed a small but significant increase in cardiac output after treatment with losartan compared to placebo and although HR was not significantly different between study days, losartan significantly increased HR compared to baseline. Although no significant increase in heart rate has been observed in acute dosing studies with losartan in patients with congestive cardiac failure, it is interesting that an increase in HR was noted in studies performed in normal volunteers pre-treated with oral frusemide (Doig JK et al, 1995). This could possibly represent a reflex vagal withdrawal response to an acute reduction in afterload or a direct effect of losartan on the autonomic nervous system.

In terms of the methodology employed to measure pulmonary haemodynamic changes, we have used the pulmonary acceleration time as a measure of mean pulmonary artery pressure which we have shown to be reproducible in both normal volunteer (Lipworth BJ et al, 1994) and patient studies (Cargill RI et al, 1996) and has been shown to have a good correlation with catheter derived measures in both our

own hands and those of other workers (Dabestani A et al, 1987, Matsuda M et al, 1986) although it is generally accepted that the pulmonary acceleration time cannot be used to precisely measure MPAP in a patient population (Dabestani A et al, 1987). We have previously employed these methods to study the pulmonary vascular effects of vasoconstrictors (Lipworth BJ et al, 1994) and vasodilators (Cargill RI et al, 1995) giving results which concur well with invasive studies of the same agents (Segel N et al, 1960, Cody RJ et al, 1986) although some studies have shown a poor correlation between changes in pulmonary acceleration time and catheter measured MPAP (Beard JT et al, 1991, Chow LC et al, 1988). Doppler echocardiography is also a well validated and reproducible measure of cardiac output (Huntsman LL et al, 1983). We do not think that the observed changes in TPR are likely to be confounded by alterations in wedge pressure since in cor pulmonale the increase in resistance is confined to the precapillary vasculature.

In addition to beneficial pulmonary haemodynamic changes, the inclusion of a placebo limb has allowed us to examine the effects of ANG II receptor blockade on plasma aldosterone levels. Plasma aldosterone is known to increase following the assumption of the upright posture and decrease following resumption of the supine posture (Tuck ML et al, 1975, Williams GH et al, 1972), an effect thought to be due to changes in both aldosterone secretion and clearance (Bougas J et al, 1964). As expected with supine rest plasma aldosterone levels fell on both study days with falls on the placebo day similar to those seen in normal volunteers (Tuck ML et al, 1975). In addition however, treatment with losartan appears to have additional beneficial effects with plasma aldosterone significantly lower 4 hours after dosing. This reduction in plasma aldosterone is likely to reflect a reduction in ANG II mediated aldosterone biosynthesis and secretion and although ACE-inhibitors have been shown to have a similar effect, ANG II blockers may theoretically produce greater aldosterone suppression since bradykinin may indirectly potentiate aldosterone release

(Rosolowsky LJ et al, 1992). By lowering plasma aldosterone, ANG II receptor blockade may act to prevent excessive salt and water retention, which maybe an important precipitating factor in acute exacerbations of cor pulmonale (Anand IS et al, 1992). The RAAS also has significant trophic effects on vascular and cardiac muscle (Pratt RE et al, 1993), whether lowering aldosterone levels and inhibiting the trophic effects of ANG II is sufficient to inhibit these mitogenic effects is unknown but may be important in arresting the cardiopulmonary remodelling characterising this condition.

So to conclude, in addition to previous work demonstrating that angiotensin II receptor blockade can attenuate the acute hypoxic pulmonary vasoconstrictor response, we have now shown that the acute administration of losartan has beneficial pulmonary haemodynamic and endocrine effects without worsening systemic oxygenation. Whether manipulating the renin angiotensin aldosterone system with angiotensin converting enzyme inhibitors or angiotensin II blockade may be of therapeutic value in hypoxaemic cor pulmonale by inhibiting cardiopulmonary remodelling or reducing right ventricular afterload can only be answered by conducting large, long-term follow up studies.

CHAPTER 10

**HYPOXAEMIA AND RELEASE
OF ENDOTHELIN-1**

Thorax 1995;50:1308-1310.

10.1 SUMMARY

Secretion of the vasoconstrictor peptide endothelin-1 from vascular endothelium is increased by a variety of stimuli. Whether hypoxaemia affects plasma levels of endothelin-1 in humans is unknown but this may well be important in the haemodynamic response to hypoxaemia. We have therefore measured plasma endothelin-1 concentrations in hypoxaemic humans.

Plasma endothelin-1 was measured by specific radioimmunoassay in 10 control subjects at rest and following 30 minutes acute hypoxaemia (SaO_2 75-80%) induced by breathing a nitrogen/oxygen mixture, and in 10 patients with hypoxaemic cor pulmonale.

Plasma endothelin-1 concentration in control subjects was increased from (mean \pm standard error) 0.90 ± 0.11 pmol/l at baseline to 2.34 ± 0.34 pmol/l during hypoxaemia. In patients with cor pulmonale, plasma endothelin-1 concentration was 2.96 ± 0.34 pmol/l, elevated in comparison with control subjects at rest but similar to levels in controls during hypoxaemia.

Plasma levels of endothelin-1 were therefore increased by hypoxaemia in humans. The elevated levels observed in patients with cor pulmonale may be largely attributable to the effects of hypoxaemia, although the pathophysiological significance of these observations remains to be established.

10.2 INTRODUCTION

The endothelins are a group of structurally similar peptides synthesised by vascular endothelium. These peptides act via two specific receptors. Stimulation of the type A endothelin receptor (ET_A), the main subtype found on vascular smooth muscle cells (Davenport AP et al, 1995), results in profound and long-lasting vasoconstriction whilst stimulation of the type B receptor (ET_B), causes release of vasodilator metabolites and transient vasodilatation (Bigaud M et al, 1992). Interestingly however, in rat pulmonary resistance vessels *in vitro*, ET_B activation may be important in mediating endothelin-1 induced vasoconstriction (MacLean MR et al, 1994). In humans, endothelin-1 is the most potent vasoconstrictor substance known through its relatively selective activation of the ET_A receptor (Arai H et al, 1990). A role in the maintenance of vascular tone is suspected but this remains speculative at present.

Synthesis of this peptide is increased by a number of humoral and physical stimuli, including hypoxia, which may be pathophysiologically relevant (Haynes WG et al, 1993). *In vitro* studies have shown that acute hypoxia increases endothelin-1 production by human endothelial cells (Kourembanas S et al, 1991) whilst in experimental animals *in vivo*, hypoxia increases plasma concentrations of endothelin-1 (Shirakami G et al, 1991). The effect of hypoxaemia on endothelin-1 in humans is unknown but may be relevant in the cardiovascular adaptations to hypoxaemia and the circulatory abnormalities seen in conditions like cor pulmonale.

We have therefore studied the effects of hypoxaemia on plasma endothelin-1 levels in healthy subjects and also in patients with hypoxaemic cor pulmonale.

10.3 METHODS

Subjects

Normal controls: Ten young male volunteers, age (mean \pm standard error) 28.1 ± 2.2 years were studied. None were taking prescribed medication and all had normal clinical history and examination, 12-lead electrocardiogram, echocardiogram and haematological and biochemical screen and forced expiratory volume in 1 second (FEV_1) $>90\%$ predicted.

Cor pulmonale patients: Ten (6 male, 4 female) patients age 73.4 ± 1.7 years with clinically stable cor pulmonale secondary to chronic obstructive pulmonary disease were studied. All had obstructive pattern spirometry (FEV_1 / forced vital capacity (FVC) $<70\%$), arterial hypoxaemia while breathing air ($PaO_2 < 8.0$ KPa), and had or gave a history of having peripheral oedema despite normal left ventricular function (on echocardiogram or radionuclide ventriculography), normal renal function (serum creatinine < 120 mmol/L) and normal serum albumin (> 35 g/l). Patients with other significant cardiovascular disease were excluded.

In this group, FEV_1 in litres was 0.75 ± 0.06 (range 0.36-1.11), FEV_1 as % of predicted 33.8 ± 5.2 (range 19-61), PaO_2 on air 6.30 ± 0.34 KPa (range 4.35-7.80) and $PaCO_2$ on air 6.17 ± 0.50 KPa (range 3.84-8.80).

Study design

Informed consent was obtained to the protocol previously approved by the Tayside Committee for Medical Research Ethics. Baseline venous blood samples were after 30 minutes supine rest while breathing air. Control subjects only were then rendered hypoxaemic (steady state SaO_2 75-80% measured by pulse oximetry) for 30 minutes by breathing a variable nitrogen/oxygen mixture before taking a further blood sample.

Measurements

Levels of endothelin-1 were measured as previously described. The lower limit of detection was 0.4 pmol/l and the intra-assay co-efficient of variation was 4.5%. The anti-endothelin antibody used was 100% specific for endothelin-1 and had cross-reactivity of 96% with endothelin-3 and 7% with proendothelin.

Data Analysis

After testing for normality of distribution, between group comparisons were made by analysis of variance followed by Duncan's multiple range testing with $p < 0.05$ considered significant [8]. Results are expressed as mean \pm standard error with 95% confidence intervals (CI) for significant differences.

10.4 RESULTS

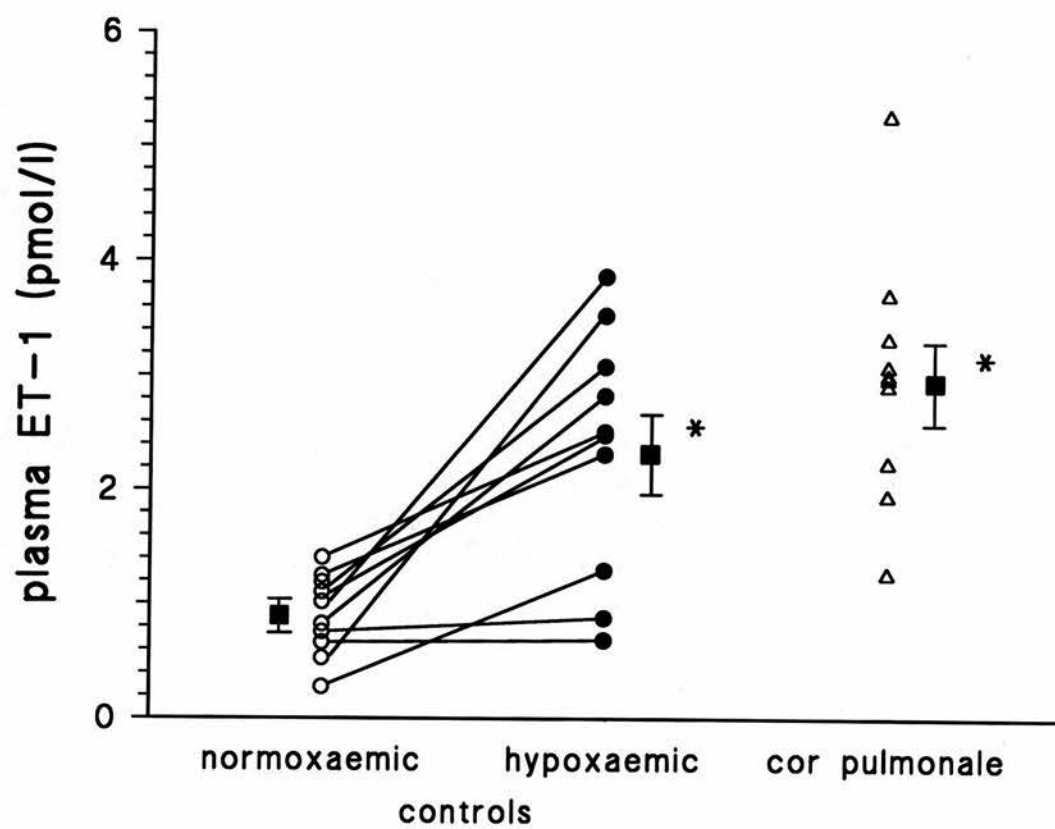
Plasma endothelin-1 concentration in normoxaemic controls was 0.90 ± 0.11 pmol/l. After 30 minutes hypoxaemia, plasma endothelin-1 increased significantly to 2.34 ± 0.34 pmol/l (95% CI for mean difference 0.41, 2.48). In patients with cor pulmonale, plasma endothelin-1 was 2.96 ± 0.34 pmol/l, significantly greater than control subjects when normoxaemic (95% CI for mean difference 1.02, 3.09) but not when hypoxaemic.

Plasma endothelin-1 therefore increased 2.6 fold in response to hypoxaemia in controls and was elevated 3.3 fold in patients with cor pulmonale compared with normoxaemic controls. Results from individual subjects with sample means are depicted in Figure 10.1.

FIGURE LEGEND

Figure 10.1. Hypoxaemia and release of endothelin-1.

Levels of endothelin-1 in $n = 10$ controls when normoxaemic (●) and following 30 minutes hypoxaemia (○), and in $n = 10$ patients with cor pulmonale (Δ). Boxes indicate sample means with standard error bars and asterisks (*) indicate values significantly ($p < 0.05$) different from normoxaemic controls.



10.5 DISCUSSION

These findings indicate that hypoxaemia increases plasma levels of endothelin-1 in humans. In patients with cor pulmonale, endothelin-1 was increased to levels comparable to those in normal subjects rendered acutely hypoxaemic. It is interesting to note that baseline levels in controls were similar to those observed in other series (Stewart DJ et al, 1991) and that the increase following hypoxaemia and in patients with cor pulmonale is comparable to the rise seen in normal subjects at high altitude (Goerre S et al, 1995).

These observations in humans are largely confirmatory of *in vitro* and animal studies which have shown hypoxia to be a potent stimulus for endothelin-1 synthesis and gene expression (Kouremabanas S et al, 1991, Shirikami G et al, 1991). In normal humans, Therkelson *et al* found that 15 minutes of hypoxaemia caused only a small increase in plasma endothelin-1 which was not statistically significant, perhaps due to the shorter duration of the stimulus (Therkelson K et al, 1994). Abnormally high levels of endothelin-1 have been described in a series of patients with pulmonary hypertension of varying aetiology, not all of whom were hypoxaemic (Stewart DJ et al, 1991) and thus other stimuli may also be implicated. In the present series, by excluding subjects with other significant cardiovascular diseases (e.g. hypertension, congestive heart failure) we feel hypoxaemia is the most significant stimulus responsible for increased endothelin-1 levels in cor pulmonale. The *in vitro* evidence would suggest that the elevated endothelin-1 levels observed were due to increased synthesis although as endothelin-1 clearance mechanisms in humans have not been fully characterised (Haynes WG et al, 1993), the possibility of decreased removal cannot be discounted.

The potent vasoactive properties of endothelin-1 may also be responsible for some of the circulatory abnormalities seen during hypoxaemia although plasma levels may not accurately reflect local concentrations and hence vasoconstrictor activity. Whether

endothelin-1 acts as a mediator of acute hypoxic pulmonary vasoconstriction is unknown. Support for this hypothesis might be drawn from studies using endothelin receptor blockers which can in rats attenuate acute hypoxic pulmonary vasoconstriction (Oparil S et al, 1995) and prevent development of pulmonary hypertension following chronic hypoxia (Eddahibi S et al, 1995). These drugs now need to be tested in humans where it is likely that endothelin-1 plays a significant role in the cardiovascular response to hypoxaemia. Manipulation of the endothelin system may therefore be a useful measure in patients with hypoxaemic lung disease, either to prevent or treat the cardiopulmonary consequences of chronic hypoxaemia.

CHAPTER 11

**CARDIOPULMONARY EFFECTS OF
ENDOTHELIN-1 IN MAN**

Cardiovascular Research 1997;33:378-386.

11.1 SUMMARY

Endothelin-1 levels are elevated in a number of conditions characterised by impaired cardiovascular performance and abnormal vasoconstriction such as congestive cardiac failure and primary and secondary pulmonary hypertension. The aim of the present study was to assess the effects of the vasoconstrictor peptide endothelin-1 on pulmonary and systemic haemodynamics and cardiovascular performance in normal man.

Ten healthy male volunteers were studied on two occasions in a randomised, double-blind, placebo controlled, cross-over study and received systemic infusions of either endothelin-1 (0.75, 1.5 and 3 pmol.kg⁻¹.min⁻¹ for 30 mins each) or saline placebo. Systemic and pulmonary haemodynamic parameters were monitored non-invasively by pulsed-wave Doppler, as were parameters of left and right ventricular diastolic filling and inotropic state. Effects on renin-angiotensin and natriuretic peptide system activity were also measured.

Endothelin-1 infusion produced dose-related falls in heart rate, stroke volume and cardiac output. Systemic vascular resistance (SVR) increased from 1156±57 to 1738±115 dynes.s.cm⁻⁵, and total pulmonary vascular resistance (TPR) increased from 142±12 to 329±22 dynes.s.cm⁻⁵. Endothelin-1 caused significant impairment of left and right ventricular diastolic filling, even at a low dose which had no pulmonary or systemic pressor effects. Electromechanical and Doppler acceleration indices of inotropic state were also significantly impaired. Activity of the renin-angiotensin system was suppressed by endothelin-1 whilst plasma levels of atrial natriuretic peptide (ANP) were unchanged.

Thus, in addition to systemic and pulmonary pressor effects our results suggest that endothelin-1 impairs overall cardiovascular performance by causing diastolic dysfunction and acting as a negatively inotropic agent. These effects were associated with compensatory changes in the renin-angiotensin system.

11.2 INTRODUCTION

Endothelial function is an important regulator of vascular tone although the physiological roles of the endothelium-derived relaxing and constricting factors and their functional antagonism remain the subject of much ongoing research effort. In terms of vasoconstriction, the family of peptides known as the endothelins have emerged as being of major importance. These are a group of structurally similar peptides synthesised by vascular endothelium which act via two specific receptors. Stimulation of the type A endothelin receptor (ET_A) results in a gradual onset but long-lasting vasoconstriction whilst stimulation of the type B receptor (ET_B), causes release of vasodilator metabolites and transient vasodilatation but may also be involved in the vasoconstrictor response (Haynes WG et al, 1993). ET_A receptors are the main subtype found on vascular smooth muscle cells (Davenport AP et al, 1995) and have also been localised to the heart and adrenal gland (Davenport AP et al, 1989). *In vitro*, endothelin-1 is the most potent vasoconstrictor substance known through its relatively selective activation of the G-protein-linked ET_A receptor (Yangisawa M et al, 1988) as well as being the most abundant endothelin isoform in human plasma (Suzuki N et al, 1989).

Release of endothelin-1 from endothelial cells is stimulated by a variety of physical and chemical stimuli *in vitro* (Haynes WG et al, 1993) whilst in humans *in vivo*, hypoxia and angiotensin II increase plasma concentrations of endothelin-1 (Cargill RI et al, 1995, Good JM et al, 1994). Abnormalities of circulating endothelin-1 concentrations have also been observed in a number of conditions characterised by abnormal vasoconstriction such as hypertension (Kohn M et al, 1990), congestive heart failure (CHF) (Cody RJ et al, 1992), primary pulmonary hypertension (PPH) (Stewart DJ et al, 1991), and hypoxaemic cor pulmonale (Cargill RI et al, 1995), raising the possibility that endothelin-1 may play a pathogenic role in both cardiovascular and cardiopulmonary disease. As the physiological effects of

endothelin-1 in man have not been fully studied, a precise understanding of this role remains elusive.

Although in previous in vivo studies in man, endothelin-1 increased systemic blood pressure (Vierhapper H et al, 1990) and caused renal vasoconstriction (Rabelink TJ et al, 1994) the effects of endothelin-1 on the pulmonary vasculature and cardiovascular performance are not known. Furthermore, the design of some studies (Vierhapper H et al, 1990) has not allowed for the prolonged action of endothelin-1 and as such, may have produced misleading information.

We have therefore evaluated the effects of systemic endothelin-1 administration in healthy volunteers. This study documents effects on systemic and pulmonary haemodynamics, diastolic ventricular function, inotropicity and neurohormonal activation.

11.3 METHODS

Subjects

Ten healthy, non-obese, male volunteers, age (mean \pm SEM) 26.1 ± 1.4 years were studied. All had normal clinical history and examination, 12-lead electrocardiogram, echocardiogram and haematological and biochemical screen. Subjects refrained from alcohol, tobacco and caffeine for at least 12 hours and had taken no medications for at least one month before the study. Informed consent was obtained to the study protocol previously approved by the Tayside Committee for Medical Research Ethics and this investigation conforms with the principles outlined in the Declaration of Helsinki.

Study design

Subjects were studied at the same time of day on two occasions at least one week apart in a randomised, double blind, cross-over design. Subjects were randomised prior to commencement of the study with both subject and echocardiographer (DGK) blinded with respect to the infusate. After completion of the final study day and of all measurements and calculations the active and placebo days were revealed to allow statistical analysis to be performed. On arrival at the clinical laboratory, intravenous cannulae were sited in both forearms for infusion (left) and blood sampling (right). Subjects then remained supine, turned slightly on the left side throughout the study. After allowing at least 30 minutes rest to achieve baseline haemodynamic parameters, infusion of either pharmaceutical grade human endothelin-1 (Clinalfa AG; Laufelfingen, Switzerland) or identical volume placebo (0.9% saline) was commenced in random order. Endothelin-1 was infused at $0.75 \text{ pmol.kg}^{-1}.\text{min}^{-1}$ for 30 minutes, then increased to $1.5 \text{ pmol.kg}^{-1}.\text{min}^{-1}$ for 30 minutes and then to $3.0 \text{ pmol.kg}^{-1}.\text{min}^{-1}$ for a further 30 minutes. Infusions were then discontinued and subjects monitored for a further 60 minutes. Measurements were made at baseline, at the end of each infusion dose period and 30 and 60 minutes after stopping the infusion.

Measurements

Pulmonary and systemic haemodynamic parameters and inotropic and diastolic indices were measured as previously described. The short term coefficients of variability (CV) for the Doppler measurements in this study were; SV 4.9%, CO 9.3%, MPAP 4.5%, mitral $E_{v_{max}}$ 12.2%, mitral $A_{v_{max}}$ 17.2%, transmitral E/A ratio 12.4%, left ventricular IVRT 13.8%, tricuspid $E_{v_{max}}$ 8.8%, tricuspid $A_{v_{max}}$ 14.2%, transtricuspid E/A ratio 14.8%, right ventricular IVRT 29.1%, Acc_{peak} 18.7%, Acc_{mean} 12.5% and QS_2I 3.5%. Venous blood samples for measurement of serum electrolytes, PRA, aldosterone and atrial natriuretic peptide were collected and analysed as previously described. The intra-assay CV for these assays were; PRA 7.6%, aldosterone 8.3%, ANP 8.0%.

Data Analysis

Comparisons were made between active and placebo treatments for each treatment by repeated measures analysis of variance (ANOVA). Where the overall ANOVA was significant, Duncan's multiple range testing was used to determine differences at individual time points. A p value of less than 0.05 was considered significant and results are expressed as means \pm SEM.

11.4 RESULTS

There were no significant adverse effects during the study although one subject felt transiently nauseous and sweaty during maximal rate infusion of endothelin-1.

Haemodynamic effects

Baseline conditions on each study day were similar for all haemodynamic parameters measured. Endothelin-1 had significant systemic pressor effects compared with placebo, where MAP was elevated following medium and high dose endothelin-1 infusion and remained significantly greater than placebo for 30 minutes after stopping the infusion (Figure 11.1a). Data for SBP and DBP are given in table 1 where the pattern of changes was similar. HR decreased during medium and high dose endothelin-1 infusion compared with placebo and remained significantly lower than placebo for 30 minutes after stopping infusions (Figure 11.1b) whilst SV was only significantly reduced during high dose endothelin-1 infusion (Table 11.1). CO also decreased during medium and high dose endothelin infusion but was not significantly different from placebo during the recovery period (Figure 11.1c). Endothelin-1 significantly increased SVR during medium and high dose infusion and remained greater than placebo after 30 minutes recovery (Figure 11.1d). In the pulmonary circulation, both MPAP and TPR were increased by medium and high dose infusion, remaining higher than placebo after 30 minutes recovery (Figures 11.1e and 11.1f).

Inotropic effects

Aortic Acc_{mean} and Acc_{peak} were similar at baseline on each study day. Both parameters decreased during endothelin-1 infusion to levels significantly lower than placebo during medium and high dose infusion for Acc_{peak} (Figure 11.2a) and at high dose only for Acc_{mean} (Figure 11.2b). Compared with placebo, QS_2I was significantly prolonged only during high dose endothelin-1 infusion (Figure 11.2c).

Lusitropic effects

All lusitropic parameters were similar at baseline on each study day. Mitral and tricuspid E/A ratio decreased during endothelin-1 infusion and were significantly different from placebo during high dose infusion only (Figure 11.3a and 11.3b). These changes were due to falls in $E_{v_{max}}$ rather than increases in $A_{v_{max}}$ (Table 11.2). IVRT was a more sensitive marker of impaired ventricular filling where in the left ventricle IVRT was prolonged by endothelin-1 even at the lowest infusion rate compared with placebo (Figure 11.3c) whilst in the right ventricle, IVRT during endothelin-1 infusion was not significantly different from placebo but was prolonged from baseline during medium and high dose infusion (Figure 11.3d).

Endocrine effects

Plasma sodium and potassium were similar at baseline on both study days and did not change significantly during either placebo or endothelin-1 infusion (Table 11.3). PRA was similar at baseline on both days and fell significantly from baseline after 90 minutes of placebo infusion (Figure 11.4a). The fall during endothelin-1 infusion was however more rapid and levels were significantly lower than baseline after low dose infusion and continued to fall up to 60 minutes after stopping infusion. Levels were significantly lower than placebo during medium and high dose endothelin-1 infusion and throughout the recovery period (Figure 11.4a). There was no difference in plasma aldosterone either at baseline or following endothelin-1 infusion compared with placebo. Levels fell significantly from baseline after 60 minutes of placebo infusion and then began to plateau (Figure 11.4b). Levels also fell following endothelin-1 infusion, reaching a significant nadir at the medium dose (Figure 11.4b). No effect on plasma ANP levels was observed during either placebo or endothelin-1 infusion (Table 11.3).

Table 11.1. Systemic haemodynamic effects of ET-1 infusion in normal man.

		<u>Time (minutes)</u>					
		<i>0</i>	<i>30</i>	<i>60</i>	<i>90</i>	<i>120</i>	<i>150</i>
SBP	Placebo	112±2.9	107±2.9	106±2.5	108±3.5	110±2.8	110±3.6
(mmHg)	Active	108±2.5	107±2.2	109±2.1	114±3.5 ^{*†}	113±1.9	112±1.9
DBP	Placebo	61±2.5	58±2.5	57±2.5	60±2.2	60±1.7	61±2.2
(mmHg)	Active	57±2.0	57±1.8	62±2.1 ^{*†}	67±3.2 ^{*†}	64±1.8 ^{*†}	64±2.4 [†]
SV	Placebo	90±4.0	89±4.2	88±3.6	86±3.2	87±4.7	89±4.4
(ml)	Active	90±3.8	88±4.4	85±4.6	81±5.0 [†]	90±4.6	90±4.3

Absolute values (mean±SEM) of systolic and diastolic blood pressure (SBP, DBP) and stroke volume (SV) during placebo and endothelin-1 infusion.

Asterisk (*) indicates significant difference from placebo at the same time point.

Cross (†) indicates significant difference from baseline during endothelin-1 infusion.

Table 11.2. Left and right ventricular filling parameters in response to endothelin-1 infusion in normal man.

All units (m.s ⁻¹)		Time (minutes)					
		0	30	60	90	120	150
Mitral	Placebo	70±4.1	69±4.3	71±4.0	68±3.4	70±3.2	68±4.0
E Vmax	Active	71±3.9	71±4.2	62±3.3 ^{*†}	59±3.4 ^{*†}	63±3.0 [†]	71±4.4
Mitral	Placebo	42±1.9	42±1.7	43±1.6	41±1.7	41±1.6	41±1.8
A Vmax	Active	41±1.7	43±2.0	40±1.8	40±2.4	38±1.8	42±2.7
Tricuspid	Placebo	47±1.7	45±1.2	47±1.3	46±1.3	46±1.4	46±1.2
E Vmax	Active	46±2.1	46±2.1	45±1.8	43±1.7	42±0.7 ^{*†}	43±1.0 [†]
Tricuspid	Placebo	31±1.0	30±1.1	31±1.2	30±0.9	32±1.1	31±0.8
A Vmax	Active	30±0.9	32±1.4	31±1.0	31±1.5	29±0.9	29±1.3

Absolute values (mean±SEM) of transmitral and transtricuspid E and A wave maximal velocity during placebo and endothelin-1 infusion.

Asterisk (*) indicates significant difference from placebo at the same time point.

Cross (†) indicates significant difference from baseline during endothelin-1 infusion.

Table 11.3. Serum electrolytes and ANP in response to ET-1 infusion in normal man.

		<u>Time (minutes)</u>					
		<i>0</i>	<i>30</i>	<i>60</i>	<i>90</i>	<i>120</i>	<i>150</i>
Na (mmol.l ⁻¹)	Placebo	138±0.7	138±0.6	138±0.8	138±0.6	136±0.8	138±1.0
	Active	137±0.7	138±0.7	137±0.9	137±0.8	137±0.6	138±0.6
K (mmol.l ⁻¹)	Placebo	4.0±0.08	4.1±0.04	4.1±0.06	4.1±0.05	4.0±0.06	4.1±0.08
	Active	4.1±0.07	4.2±0.11	4.1±0.08	4.2±0.06	4.2±0.08	4.2±0.09
ANP (pmol.l ⁻¹)	Placebo	4.82±1.53	4.61±0.59	4.36±0.43	4.05±0.49		
	Active	5.57±0.68	5.97±0.43	5.71±0.79	6.00±0.71		

Absolute values (mean±SEM) of serum sodium (Na), potassium (K) and atrial natriuretic peptide (ANP) during placebo and endothelin-1 infusion. There were no significant differences between endothelin-1 and placebo treatments or with time.

FIGURE LEGEND

Figure 11.1a, b, c, d, e and f. Systemic and pulmonary haemodynamic changes in response to ET-1 (■) and placebo (○) infusions in normal man.

The doses of endothelin-1 infused are shown in diagrammatic form on the x-axis. Endothelin-1 was infused at $0.75 \text{ pmol.kg}^{-1}.\text{min}^{-1}$ for 30 minutes, then increased to $1.5 \text{ pmol.kg}^{-1}.\text{min}^{-1}$ for 30 minutes and then to $3.0 \text{ pmol.kg}^{-1}.\text{min}^{-1}$ for a further 30 minutes.

Asterisk (*) indicates significant difference from placebo at the same time point.

Cross (†) indicates significant difference from baseline during endothelin-1 infusion.

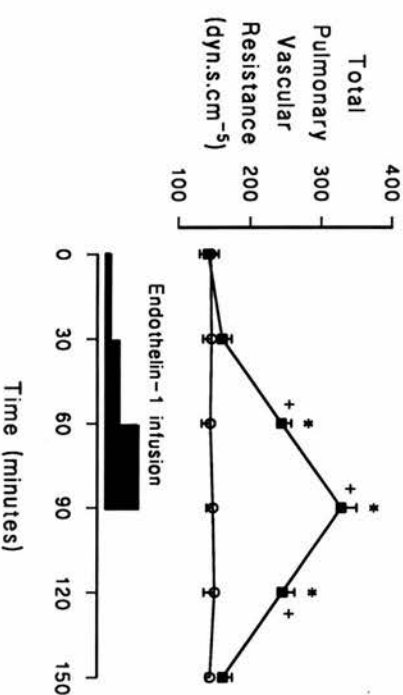
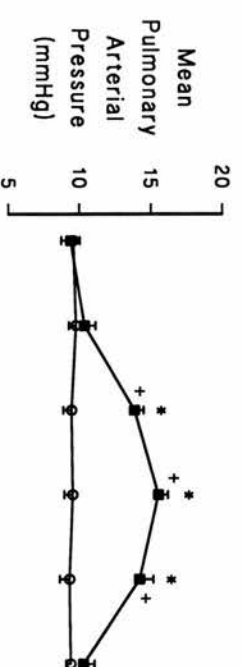
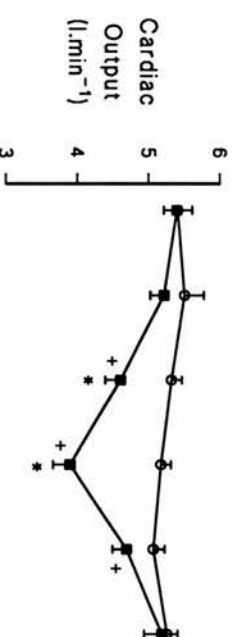
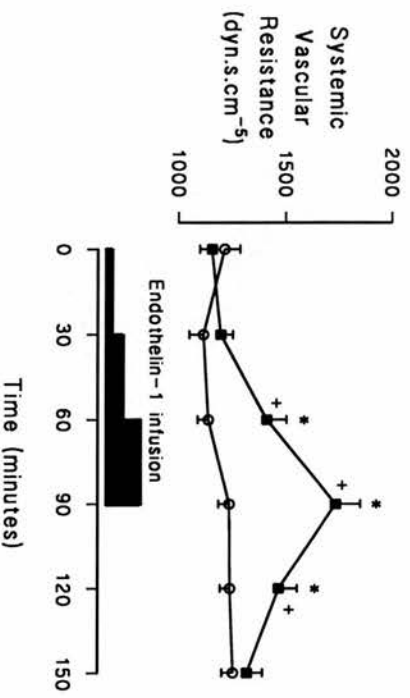
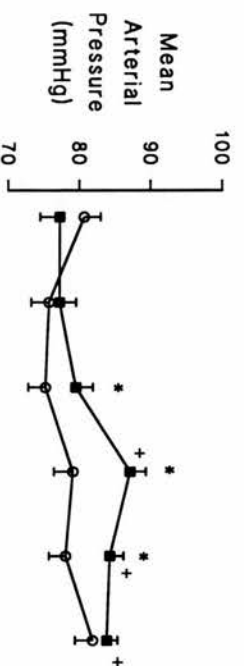
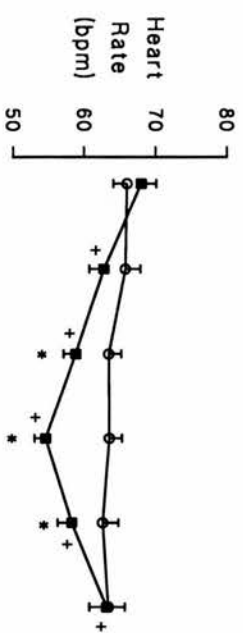


FIGURE LEGEND

Figure 11.2a, b and c. Changes in inotropic indices in response to endothelin-1 (■) and placebo (○) infusions in normal man.

The doses of endothelin-1 infused are shown in diagrammatic form on the x-axis. Endothelin-1 was infused at $0.75 \text{ pmol.kg}^{-1}.\text{min}^{-1}$ for 30 minutes, then increased to $1.5 \text{ pmol.kg}^{-1}.\text{min}^{-1}$ for 30 minutes and then to $3.0 \text{ pmol.kg}^{-1}.\text{min}^{-1}$ for a further 30 minutes.

Asterisk (*) indicates significant difference from placebo at the same time point.

Cross (†) indicates significant difference from baseline during endothelin-1 infusion.

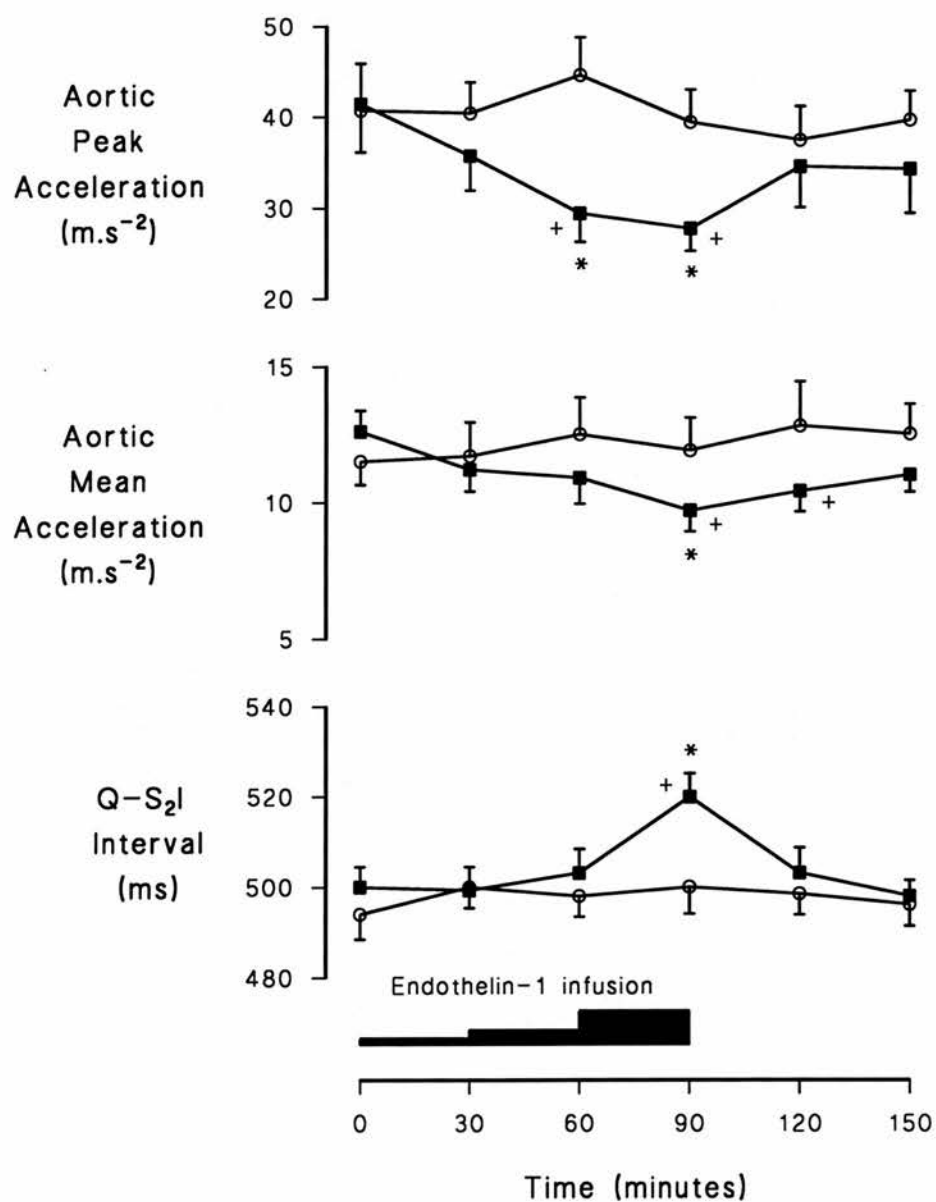


FIGURE LEGEND

Figure 11.3a, b, c and d. Echo-Doppler parameters of left (LV) and right (RV) ventricular filling in response to endothelin-1 (■) and placebo (○) infusions in normal man.

The doses of endothelin-1 infused are shown in diagrammatic form on the x-axis. Endothelin-1 was infused at $0.75 \text{ pmol.kg}^{-1}.\text{min}^{-1}$ for 30 minutes, then increased to $1.5 \text{ pmol.kg}^{-1}.\text{min}^{-1}$ for 30 minutes and then to $3.0 \text{ pmol.kg}^{-1}.\text{min}^{-1}$ for a further 30 minutes.

Asterisk (*) indicates significant difference from placebo at the same time point.

Cross (†) indicates significant difference from baseline during endothelin-1 infusion.

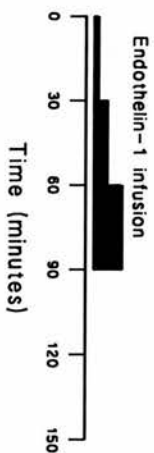
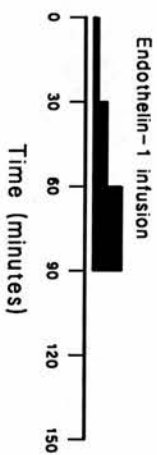
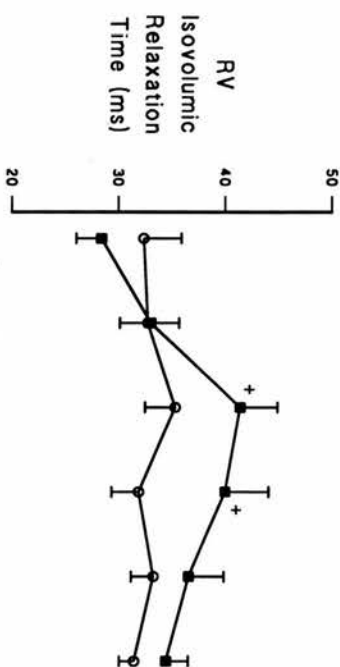
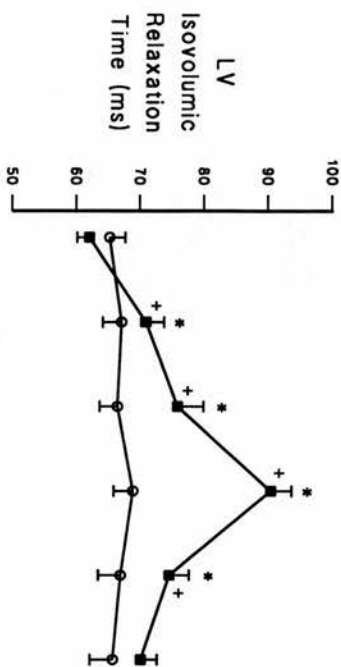
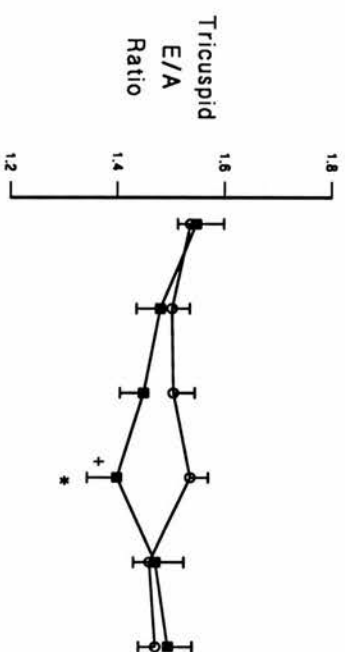
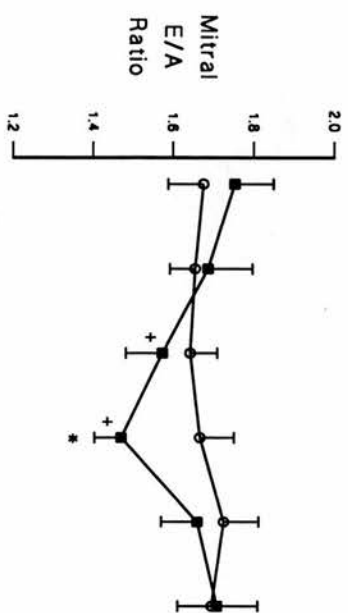


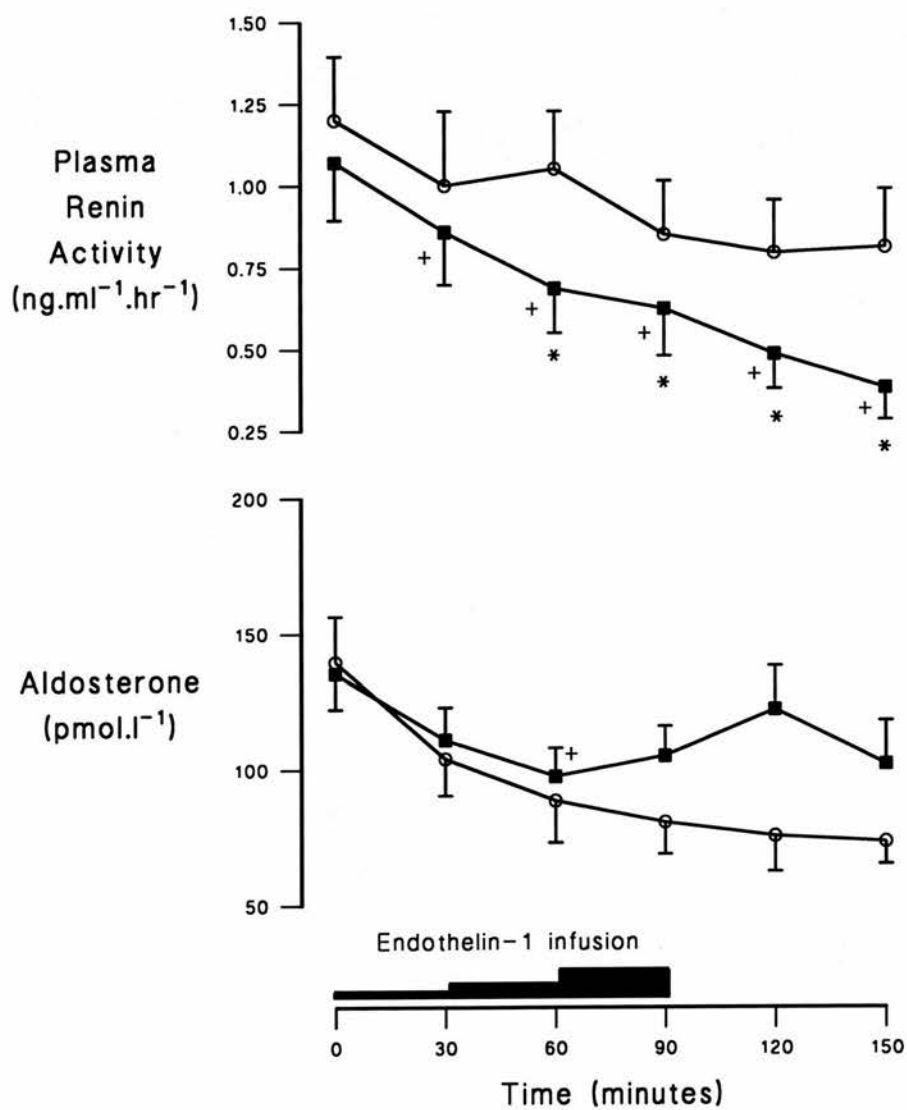
FIGURE LEGEND

Figure 11.4a and b. Changes in renin-angiotensin system activity in response to endothelin-1 (■) and placebo (○) infusions in normal man.

The doses of endothelin-1 infused are shown in diagrammatic form on the x-axis. Endothelin-1 was infused at $0.75 \text{ pmol.kg}^{-1}.\text{min}^{-1}$ for 30 minutes, then increased to $1.5 \text{ pmol.kg}^{-1}.\text{min}^{-1}$ for 30 minutes and then to $3.0 \text{ pmol.kg}^{-1}.\text{min}^{-1}$ for a further 30 minutes.

Asterisk (*) indicates significant difference from placebo at the same time point.

Cross (†) indicates significant difference from baseline during active infusion.



11.5 DISCUSSION

Although endothelin-1 was initially characterised as a vasoconstrictor, it is clear from the present study that in addition to pressor effects, endothelin-1 may also have direct effects on myocardial function in man. In comparison with animal studies (Goetz KL et al, 1988), these effects were observed using relatively low doses of endothelin-1, although just exceeding the previously highest reported dose in humans (Vierhapper H et al, 1990). We have administered endothelin-1 exogenously in a range of doses, with the lowest dose achieving levels of endothelin-1 found in pathophysiological states whilst the higher doses will have achieved so called "pharmacological" concentrations. Unlike previous studies we have not measured plasma endothelin-1 levels as this appears highly variable between individuals during infusions of endothelin-1 (Vierhapper H et al, 1990) and as endothelin-1 has paracrine rather than endocrine actions, plasma levels may not be comparable with levels measured in pathological states. In these conditions plasma levels are likely to represent "spillover" with much higher levels of endothelin-1 found abluminally. It is for these reasons that our dose range included infusions that were known to achieve pathophysiological concentrations of endothelin-1 and greater. The relatively longer infusion times in the present investigation have also allowed a clearer definition of the dose related effects of endothelin-1 than would be obtained with shorter dose increments.

The parameters in this study were measured non-invasively due to ethical considerations and as such have associated limitations. Although our indices of inotropicity and lusitropicity have previously been validated (Sabbah HN et al, 1986,

Ahmed SS et al, 1972, Rokey R et al, 1985) these measures can be affected to greater or lesser degrees by loading conditions and we have alluded to this in the text. Doppler echocardiography was used to measure haemodynamic changes in the pulmonary circulation. Pulmonary acceleration time, which we have shown to be reproducible in our own hands, was used to estimate mean pulmonary artery pressure which has been shown to have a good correlation with catheter derived measures, although in a patient population it cannot be accurately used to precisely estimate pulmonary artery pressure (Dabestani A et al, 1987, Matsuda M et al, 1986). Although estimation of changes in pulmonary artery pressure over time may be accurate, some caution should be used when taking these values for further calculations. We looked at two measures of pulmonary vasoconstriction changes in MPAP and TPR. A limitation of this methodology is that TPR neglects the downstream pressure of the pulmonary circulation, namely left atrial pressure, as conventionally assessed by pulmonary capillary wedge pressure (PCWP). Although this may be increased if left ventricular function is impaired, as was the case in this study, the magnitude of the changes observed in the pulmonary vascular bed suggest an effect on the pre-capillary vasculature. With respect to Doppler measures of cardiac output, these are accepted as a relatively accurate measure which can be made in most patients (Huntsman LL et al, 1983), the major source of error is generally accepted as measurement of the annular diameter and this would be mitigated by the cross-over design of the study. In addition it remains difficult to separate primary from secondary effects of endothelin-1 on cardiopulmonary performance using this protocol although some of the data does support direct effects on the heart.

The haemodynamic effects of endothelin-1 in man are not well characterised although our haemodynamic data agree with findings from animal studies (Goetz KL et al, 1988). Our study confirms that endothelin-1 is a potent in vivo systemic vasoconstrictor and it is interesting to note that MAP and SVR were elevated compared to baseline for at least 30 minutes after stopping the infusion indicating prolonged vasoconstriction. This is in keeping with in vitro data (Kiowski W et al, 1991) and with the observation that although plasma levels fall rapidly after stopping endothelin-1 infusion, the increase in blood pressure is maintained (Vierhapper H et al, 1990). The relative bradycardia and fall in CO during endothelin infusion may have been due to a vagal reflex response to systemic vasoconstriction, myocardial ischaemia or due to a direct effect on the conducting system of the heart.

Most previous studies have neglected the action of endothelin-1 on pulmonary haemodynamics. We have tried to characterise these using non-invasive techniques which we have previously employed to study the pulmonary vascular effects of vasoconstrictors (Lipworth BJ et al, 1994) and vasodilators (Cargill RI et al, 1995), giving results which concur well with invasive studies of the same agents (Segel N et al, 1960, Cody RJ et al, 1986). We have measured TPR and not pulmonary vascular resistance (PVR) and as such have neglected the downstream pressure of the pulmonary circulation. Our results suggest that endothelin-1 impairs diastolic function and acts as a negatively inotropic agent, and as such have reasons to believe that left atrial pressure did increase in these subjects. Although we cannot conclude that PVR was increased in our subjects the magnitude of the changes in the pulmonary vascular bed is consistent with a vasoconstrictor effect for endothelin-1, an effect that would

be predicted from in vitro and animal studies (Goetz KL et al, 1988, Crawley D et al, 1989). At a receptor level, although still attributable to endothelin-1, some debate exists about the role activation of the ET_B receptor plays in producing pulmonary vasoconstriction (MacLean MR et al, 1994).

Our findings also support the hypothesis that endothelin-1 has negative inotropic effects and impairs ventricular filling in humans and is similar to the findings of previous animal studies (Ohno M et al, 1994). We have used echo-Doppler parameters and systolic time intervals to assess left ventricular contractility as both QS₂I and Acc_{peak} are thought to be relatively independent of loading conditions. In this respect in vitro and in vivo work have shown that changes in QS₂I reflect inotropic influences and load conditions only have a proportionately small effect on this interval (Li Q et al, 1993) although conflicting data exists regarding Doppler derived parameters of ascending aortic flow (Bennet ED et al, 1984, Bedotto JB et al, 1989). Studies examining effect of afterload on transmitral flow patterns have been hampered by difficulties involving sympathetic and neurohormonal activation, however, the general consensus is that afterload decreases early filling velocity and E/A ratio (Zile MR et al, 1993). The IVRT also varies with changes in afterload and prolongation of the IVRT is directly related to the natural logarithm of aortic closing pressure (Thomas JD et al, 1992). Although the changes in IVRT and our Doppler indices of left and right ventricular function can be explained in part by increases in afterload it is important to note that changes in left ventricular IVRT were observed at low dose endothelin-1 infusion which had no significant pressor effects. This suggests

that endothelin-1 may impair ventricular filling via a direct effect on the myocardium as well as by increasing afterload.

Thus in addition to a reflex response to vasoconstriction the observed fall in CO and SV may also be due a direct effect of endothelin-1 on the myocardium possibly mediated through the ET_A receptor which is found on cardiac muscle (Davenport AP et al, 1989) although a secondary effect from myocardial ischaemia remains a possibility, despite the absence of clinical or ECG evidence of ischaemia in these subjects. Alternatively, endothelin-1 by directly affecting myocyte calcium handling could lead to inefficient excitation-contraction coupling, an effect observed in vitro (Kohmoto O et al, 1993). The changes in IVRT observed were of similar magnitude to abnormalities associated with pulmonary heart disease (Marangono S et al, 1992) and hypertensive heart disease (Grossman W, 1991). Whether endothelin-1 contributes to the ill-defined syndrome of diastolic heart failure is also unknown.

In terms of effects on the renin-angiotensin system (RAS), endothelin-1 has been variously reported to have stimulatory and inhibitory actions (Haynes WG et al, 1993). In vitro studies would indicate that endothelin-1 inhibits renin release (Takagi M et al, 1988) and stimulates aldosterone release in cell culture (Cozza EN et al, 1989) although in humans in vivo, this effect may be offset by renal vasoconstriction (Rabelink TJ et al, 1994) increasing renin release and hence aldosterone, as no effect on these parameters has been reported (Vierhapper H et al, 1990, Rabelink TJ et al, 1994). The findings of the present study are therefore of interest where the inclusion of a placebo limb allows the prolonged, time dependent fall in parameters of RAS

activity to be considered. We observed PRA to fall in comparison with placebo after medium dose infusion, an effect which continued throughout the remainder of the study even when haemodynamic parameters had normalised. This therefore provides indirect evidence for a direct effect on renin production rather than a phenomenon secondary to renal vasoconstriction. The pattern with regard to aldosterone was different where although not significantly different from placebo, there was a biphasic response during endothelin-1 infusion. This apparent dissociation of components of the RAS may be related to increases in ACTH-mediated effects on aldosterone secretion. In addition despite the increase in cardiac afterload, no change in plasma ANP concentration was observed.

Contrary to a previous study in humans (Vierhapper H et al, 1990), we found no significant effects on plasma electrolytes, a finding in agreement with animal studies (Goetz KL et al, 1988). The possibility that mild haemolysis was responsible for this finding has been raised (Vierhapper H et al, 1990) and thus different peptide or infusion preparation practices may have been responsible.

We have shown that endothelin-1, in addition to potent pressor effects has adverse effects on both inotropic and lusitropic parameters. These new findings may be important in considering the pathophysiological role of endothelin-1 in both cardiovascular and cardiopulmonary disease.

CHAPTER 12

**NITRIC OXIDE: AN IMPORTANT ROLE IN THE
MAINTENANCE OF SYSTEMIC AND PULMONARY
VASCULAR
TONE IN MAN**

British Journal of Clinical Pharmacology 1998;46:263-266.

12.1 SUMMARY

The aim of his study was to examine whether nitric oxide (NO) has an important role in maintaining basal vascular tone in normal man by examining the effects of nitric oxide inhibition using N^G-monomethyl-L-arginine (L-NMMA) on systemic and pulmonary haemodynamics.

10 normal male volunteers 26 ± 1.6 years were studied on two separate occasions in a double blind, placebo controlled crossover study. They were randomised to receive either a continuous infusion of L-NMMA (4mg/kg/hr) with a front loaded bolus (4mg/kg) or volume matched placebo. Pulsed wave Doppler echocardiography was used to measure cardiac output (CO), mean pulmonary artery pressure (MPAP) and hence systemic vascular resistance (SVR) and total pulmonary vascular resistance (TPR). Measurements were made prior to infusion (T_0) and after 4, 8, and 12 minutes (T_1 , T_2 and T_3).

Infusion of L-NMMA significantly increased mean arterial blood pressure (MAP), SVR and TPR and significantly reduced heart rate (HR), stroke volume (SV) and CO compared to placebo. These effects were observed at T_1 and persisted during the entire infusion period.

These results are consistent with a role for basal nitric oxide generation in the maintenance of basal systemic and pulmonary vascular tone in normal man.

12.2 INTRODUCTION

Endothelial function is known to be an important determinant of vascular tone although the physiological roles of the endothelium-derived relaxing and constricting factors and their functional antagonism remain the subject of much ongoing research effort. Much interest surrounds a potent short acting vasodilator, nitric oxide, which is generated by the vascular endothelium through the action of nitric oxide synthase (Palmer RMJ et al, 1987). Studies in animals using systemic infusions of nitric oxide synthase inhibitors such as N^G -monomethyl-L-arginine (L-NMMA) have demonstrated an increase in mean arterial pressure and systemic vascular resistance (Rees DD et al, 1989, Loeb AL et al, 1992). Evidence in humans suggesting an important role for nitric oxide generation in the maintenance of systemic vascular tone comes from studies demonstrating that infusion of L-NMMA into the brachial artery results in vasoconstriction (Vallance P et al, 1989) and systemic infusions of L-NMMA increase systemic vascular resistance (Haynes WG et al, 1993).

There is however, some debate as to the role of nitric oxide generation in the maintenance of the low pressure pulmonary circulation where conflicting results exist suggesting the possibility of species specificity (Stamler JS et al, 1994, Fineman JR et al, 1992, McMahon TJ et al, 1991, Hasunuma K et al, 1991, Nishiwaki K et al, 1992). Interestingly, studies in animals and humans have suggested that impaired endothelial relaxing activity may contribute to the pathogenesis of pulmonary hypertension (Adnot S et al, 1991, Dinh-Xuan AT et al, 1991). To date only Stamler and his colleagues have examined the effects of nitric oxide inhibition on pulmonary vascular tone in normal man in a dose ranging study (Stamler JS et al, 1994). This study examines for the first time in a placebo controlled manner the effects of nitric oxide inhibition on pulmonary vascular tone in normal man using non-invasive pulsed wave Doppler echocardiography.

12.3 METHODS

Subjects

Ten healthy, non-obese, male volunteers, age (mean \pm SEM) 26 \pm 1.6 years were studied. All had normal clinical history and examination, 12-lead electrocardiogram, echocardiogram and haematological and biochemical screen. Subjects refrained from alcohol, tobacco and caffeine for at least 12 hours and had taken no medications for at least one month before the study. Informed consent was obtained to the study protocol previously approved by the Tayside Committee for Medical Research Ethics and this investigation conforms with the principles outlined in the Declaration of Helsinki.

Protocol

Subjects were studied at the same time of day on two occasions in a randomised, double blind, cross-over design. Subjects then remained supine, turned slightly on the left side throughout the study. After allowing at least 30 minutes rest to achieve baseline haemodynamic parameters they received either a continuous infusion of L-NMMA (4mg/kg/hr) with a front loaded bolus (4mg/kg) given over two minutes or volume matched placebo (0.9% N. saline). Measurements were made prior to infusion (T_0) and after 4, 8, and 12 minutes (T_1 , T_2 and T_3).

Measurements

Systemic and pulmonary haemodynamic indices were measured as previously described. The coefficients of variability (CV) for these measurements in this study were HR: 12.8%; SV: 18.7%; CO: 14.0%; PAT 2.8%; and MAP: 7.1%.

Data Analysis

Comparisons were made between active and placebo treatments by repeated measures analysis of variance (ANOVA). Where the overall ANOVA was significant, Duncan's multiple range testing was used to determine differences at individual time points . A p value of less than 0.05 was considered significant and results are expressed as means \pm SEM and where a difference between means is quoted, the 95% confidence (CI) for this difference is given.

12.4 RESULTS

Acute administration of L-NMMA was not associated with any adverse effects during the study. Baseline conditions on each study day were similar for all haemodynamic parameters measured. L-NMMA had significant pressor effects compared with placebo, where both SVR ($p=0.02$); mean difference $479 \text{ dyne.s.cm}^{-5}$ (95% CI 87-872) and TPR ($p=0.02$); $63 \text{ dyne.s.cm}^{-5}$ (95% CI 15-110) were elevated at T_1 and these effects persisted throughout the entire infusion period (Figure 12.1). Although MAP was significantly increased during infusion of L-NMMA compared to placebo at T_1 ($p=0.03$); mean difference 5mmHg (95% CI 1-10) an effect that persisted throughout the entire infusion period, MPAP was unchanged. In addition HR ($p=0.003$); mean difference 9 bpm (95% CI 5-13) and CO ($p=0.02$); mean difference 1.74 L/min (95% CI 0.33-3.15) were significantly lower during infusion with L-NMMA compared to placebo at T_1 an effect that persisted throughout the infusion period (Table 12.1).

Table 12.1 Haemodynamic effects of L-NMMA and placebo infusions in normal man.

		T0	T1	T2	T3
HR	P	71±2	69±3	70±4	67±3
(bpm)	L	68±4	60±4 * ⁺	59±3 * ⁺	57±3 * ⁺
SV	P	102±5	104±6	101±4	111±6
(ml)	L	100±6	95±6 ⁺	91±5 ⁺	90±7 * ⁺
CO	P	6.92±0.47	6.91±0.48	6.66±0.44	7.21±0.56
(L/min)	L	6.34±0.52	5.17±0.5 * ⁺	5.10±0.41 * ⁺	5.27±0.52 * ⁺
MAP	P	87±3	89±3	89±3	87±3
(mmHg)	L	90±3	94±3 * ⁺	95±3 ⁺	94±2 * ⁺
MPAP	P	11±1	11±1	12±1	11±1
(mmHg)	L	10±1	12±1	12±2	12±1

Absolute values (mean±SEM).

Asterisk (*) indicates significant difference between infusion with L-NMMA (L) and placebo (P) at that time point.

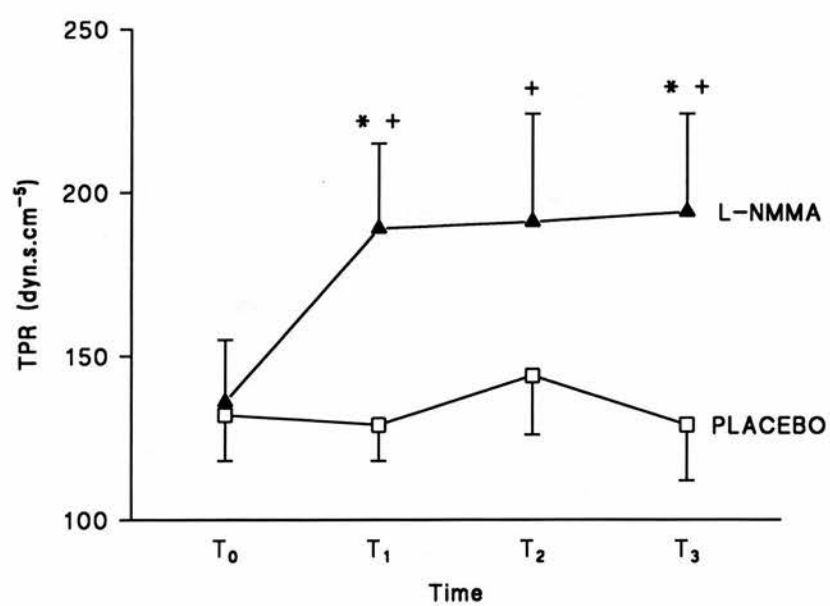
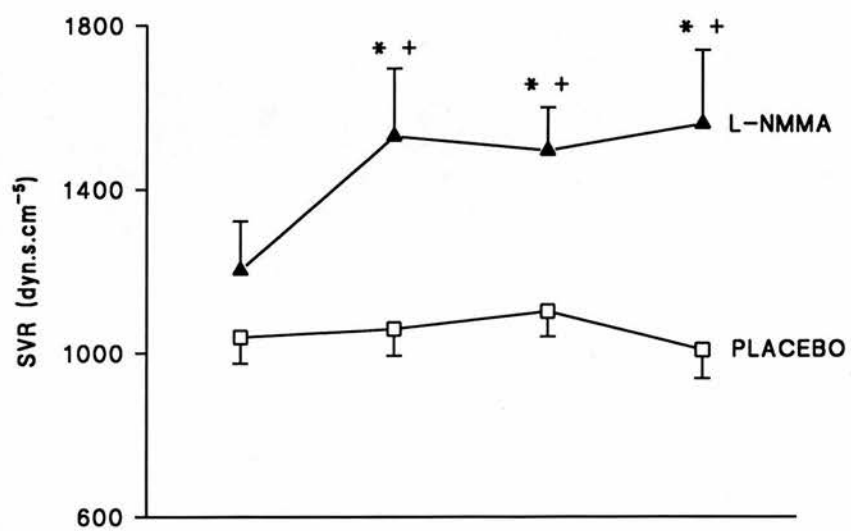
Cross (+) indicates significant difference from baseline during L-NMMA infusion.

FIGURE LEGEND

Figure 12.1. Systemic and pulmonary pressor effects of L-NMMA and placebo infusions in normal man.

Asterisk (*) indicates significant difference between infusion with L-NMMA and placebo at that time point.

Cross (+) indicates significant difference from baseline during L-NMMA infusion.



12.5 DISCUSSION

Our results suggest that basal generation of NO is important in the regulation of both systemic and normoxic pulmonary vascular tone in normal man reflected by an increase in both SVR and TPR during inhibition of nitric oxide synthesis.

The systemic effects of NO inhibition in man are well documented. Our results however suggest that in addition that resting normoxic pulmonary vascular tone is dependent on the continuous generation of nitric oxide. In this respect infusion of L-NMMA resulted in a significant increase in TPR which was sustained throughout the entire infusion period. The lack of effect on MPAP is likely to reflect the coexistent reduction in cardiac output. Although this phenomenon has been shown in some lower mammals (Fineman JR et al, 1992, McMahon TJ et al, 1991) it has not been demonstrated in either rat (Hasunuma K et al, 1991) or dog models (Nishiwaki K et al, 1992). The results of this study support the findings of Stamler et al (Stamler JS et al, 1994) who demonstrated a similar effect during a dose ranging study in normal man. In addition, our results suggest that these changes persist throughout a more prolonged infusion period.

We chose to use a dose of L-NMMA based on findings from previous in vivo studies conducted in man to insure a good profile of nitric oxide inhibition over the whole time course of the study and found comparable systemic haemodynamic effects. Previous work by Haynes et al (Haynes WG et al, 1993) examined the maximal haemodynamic responses to increments in doses of L-NMMA. He examined on

separate days doses of L-NMMA in ascending order (0.03, 0.1, 0.3, 1.0, 3.0 mg/kg) infused over 60 minutes and then 3mg/kg L-NMMA infused over 20 minutes then the same dose infused over 5 minutes via a peripheral cannula. Although, at doses >1mg/kg there was an apparent decrease in heart rate, cardiac index and an increase in peripheral vascular resistance no effect on blood pressure was observed until a dose of 3mg/kg was given over 20 minutes when there were isolated increases in both systolic and diastolic blood pressure but these did not occur consistently at similar time points. He and co-workers performed the definitive study using 3mg/kg over 4 minutes and found maximal increases in mean arterial blood pressure 10 minutes after the start of the infusion. In a dose ranging study by Stamler et al (Stamler JS et al, 1994) the haemodynamic responses to dosages of 0.01, 0.03, 0.1, 0.3 and 1.0mg/kg/min each for 3 minutes via a central venous catheter sheath were examined. Although an increase in mean arterial blood pressure was observed at 0.1mg/kg/min infused over 3 minutes no effects were observed on the pulmonary vascular bed until the maximal rate of infusion 1.0mg/kg/min given over 3 minutes. The discrepancy between effects on the systemic and pulmonary vascular beds may be explained by in vitro studies suggesting that L-NMMA is a relatively ineffective inhibitor of NO synthase in pulmonary blood vessels (Archer SL et al, 1992).

We used a front loaded infusion ie a combination of bolus with a continuous infusion to give a good overall profile of NO inhibition over the whole time course of the study. We infused an initial 4mg/kg bolus over 2 minutes (ie 2mg/kg/min for 2 minutes) with a continuous infusion of 4mg/kg/hr to ensure that we would achieve maximal NO inhibition during the period under investigation . The maximal

haemodynamic changes in this study were noted at 4 minutes and persisted throughout the entire infusion period. We cannot comment whether haemodynamic changes occurred earlier than 4 minutes since the time scale of measurements was influenced partly by the time required for signal acquisition and measurements to be made in triplicate.

In addition for safety considerations we did not feel that a dose escalation above the infusion rate used in this study would be safe because of worries over limiting systemic hypertension and worries over precipitating coronary vascular spasm. The evidence from studies suggests that L-NMMA is a relatively ineffective inhibitor of NO synthase in pulmonary blood vessels (Archer SL et al, 1992) and therefore to construct a dose response curve based on the results of previous studies would have meant doubling and quadrupling our dose of L-NMMA and exposing our patients to unwanted risks.

With respect to methodology, we have used non-invasive Doppler-echocardiography to measure haemodynamic changes in the pulmonary circulation. These non-invasive techniques have been shown to be reproducible (Lipworth BJ et al, 1994) and the close correlation between Doppler PAT and MPAP as measured by right heart catheter, is well established both in our own hands and those of other workers (Kiely DG et al, 1997, Dabestani A et al, 1987, Kitbatake A et al, 1983, Graettinger WF et al, 1987). Less, however, is known of the correlation between changes in MPAP and PAT. Using regression equations incorporating PAT, Chow et al (Chow LC et al, 1988) examined patients before and after pulmonary thromboendarterectomy and

although statistically significant the correlation between changes in PAT and catheter measures of MPAP was weak ($r=-0.41$). Beard et al (Beard JT et al, 1991), however, showed a good correlation in 11 normal subjects made hypoxic $r=-0.73$. In addition studies performed in normal man using this non-invasive methodology concur with results of invasive studies and we therefore feel that the available evidence supports our view that PAT can be used to assess changes in the pulmonary vascular bed, particularly in normal volunteers where repeated cardiac catheterisation is viewed by some including ourselves as unethical. Doppler derived measures of cardiac output have been shown to be reproducible and correlate well with invasive measures (Huntsman LL et al, 1983). The major source of error is generally accepted as measurement of the annular diameter and this would be mitigated by the cross-over design of this study.

There is a growing body of evidence suggesting that endothelial dysfunction is important in the development of hypoxic pulmonary hypertension. Endothelium dependent relaxation is reduced in chronically hypoxic rats (Adnot S et al, 1991) and in man in end stage chronic hypoxic lung disease endothelium dependent pulmonary artery relaxation is impaired and in addition the contractile responses to pressor stimuli increased (Dinh-Xuan AT et al, 1991). These patients are known to have elevated levels of pressor substances such as endothelin-1 (Cargill RI et al, 1995), which has been shown to be potent pressor agent in man in vivo (Kiely DG et al, 1997), and may have significantly greater pressor effects in the context of a dysfunctional endothelium.

In addition to pressor effects we also noted a reduction in HR, CO and SV. This may have been mediated via a baroreceptor reflex and increase in left ventricular afterload although a role for nitric oxide generation in the maintenance of cardiac output remains a possibility.

To conclude, we have shown that basal nitric oxide generation is important in maintaining basal vascular tone. In disease states an imbalance of constricting and relaxing factors may contribute to both systemic and pulmonary vasoconstriction. The development of therapies which are able to deliver relaxing factors such as nitric oxide to the site of endothelial dysfunction hold exciting prospects, particularly in chronic hypoxic lung disease where effective therapies are limited.

CHAPTER 13

**ELEVATED LEVELS OF NATRIURETIC PEPTIDES
IN PATIENTS WITH
PULMONARY THROMBOEMBOLISM**

Submitted Cardiovascular Research 1999.

13.1 SUMMARY

We hypothesised that levels of natriuretic peptides would be increased in patients with pulmonary thromboembolism (PTE) as a consequence of the cardiopulmonary sequelae and that measuring levels of these peptides might be of value in the management of this condition.

Venous blood samples were obtained from an unselected group of 114 patients with suspected PTE referred for ventilation-perfusion scintigraphy. B-type natriuretic peptide (BNP), atrial natriuretic peptide (ANP) and N-terminal pro-ANP (N-ANP) were measured by radioimmunoassay using commercially available kits. The scans were classified into three groups according to standard criteria (PIOPED); normal scan (N) (n=20), low/intermediate probability (L/I) of PTE (n=77) and high probability (H) of PTE (n=17). Comparisons were also made between patients with high probability scans who died (n=3) and those who survived (n=14). Values are quoted for the median and interquartile ranges. There were statistically significant differences between groups for levels of a) BNP ($p<0.001$): N=6.7pmol/l (5.6-11.9), L/I=12.5pmol/l (6.7-28.2) and H=18.5pmol/l (12.6-74.6); b) ANP ($p<0.005$): N=12.6pmol/l (7.1-16.0), L/I=19.51pmol/l (12.5-28.2) and H=19.1pmol/l (15.7-31.7) and c) N-ANP ($p<0.05$): N=177 pmol/l (119-200), L/I=302 pmol/l (152-576) and H=322 pmol/l (223-563). Levels of BNP and ANP were significantly ($p<0.05$) higher in patients with high probability scans who died than in those who survived; BNP: 91.6 pmol/l (77.5-336.2) vs 14.4 pmol/l (11.9-27.4) and ANP 32.5 pmol/l (21.7-105.5) vs 17.6 pmol/l (15.2-19.3), respectively. Levels of ANP and BNP were elevated in patients with PTE.

Measuring these peptides may help to evaluate the cardiopulmonary effects of pulmonary thromboembolism as well as identifying a high risk group who may benefit from more intensive therapy such as thrombolysis.

13.2 INTRODUCTION

The clinical diagnosis of pulmonary thromboembolism (PTE) is notoriously difficult and as a consequence much emphasis has been placed on both direct and indirect imaging of the pulmonary vascular bed. The present gold standard is pulmonary angiography but this is not widely available and the diagnosis is usually made on a combination of clinical impression and the results of a ventilation perfusion scan. Interest has recently surrounded the emergence of helical computerised tomogram scanning of the thorax as a diagnostic tool in pulmonary embolism (Remy -Jardin M et al, 1992, Teigen CL et al, 1995, Hansell DM et al, 1997) and the results of an ongoing multicentre European Trial are currently awaited. The British Thoracic Society has recently published guidelines on the diagnosis and management of PTE (BTS Standards of Care Committee, 1997). However, in addition to diagnostic difficulty there is still debate regarding the role of thrombolysis in the immediate management of large PTE (Gulba DC et al, 1994, Millar GAH et al, 1971, Goldhaber SZ et al, 1993).

In an ideal situation the clinician would like to be aided by a series of tests that not only aid in the diagnosis but also help in risk stratification such that an informed decision concerning the risk/benefit ratio of a number of treatments can be made. Recently there has been much interest in a family of peptides known as that natriuretic peptides and their potential diagnostic value in conditions such as heart failure (McDonagh TA et al, 1998, Davidson NC et al, 1996). Atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP) are secreted predominantly from the atrial and ventricular myocardium respectively in responses to changes in wall tension (Yasue H et al, 1994) and plasma levels of these peptides have been shown to be sensitive markers of left ventricular systolic dysfunction (McDonagh TA et al, 1998, Davidson NC et al, 1996). Acute pulmonary thromboembolism results in a variety of derangements of pulmonary function and a significant number patients are known to have increased right atrial and ventricular pressures and arterial hypoxaemia (McIntyre

KM et al, 1971, Szucs MM et al, 1971) all of which are known to stimulate natriuretic peptide secretion. In addition patients who die acutely of pulmonary thromboembolism are thought to have died as a consequence of right ventricular failure and a recent study has shown that right ventricular afterload stress is a major determinant of short term prognosis in patients with PTE (Kasper W et al, 1997).

We have hypothesised, therefore, that levels of natriuretic peptides may be elevated in patients with pulmonary thromboembolism as a consequence of the cardiopulmonary sequelae and that measuring levels of these peptides may help us identify high risk patients with incipient right heart failure who may be at high risk of sudden death.

13.3 METHODS

Subjects

114 unselected patients age range 20-91 years were recruited into this prospective study, approved by the Tayside Committee for Medical Research Ethics, over a 3 month period. All patients were referred by their own physician according to their clinical suspicion of pulmonary thromboembolism and each patient gave informed written consent to blood sampling. There were no exclusions from the study on the basis of underlying medical problems. Patient characteristics are shown in Table 13.1 These include those known to have medical conditions associated with elevated levels of natriuretic peptides.

Radionuclide scans

Ventilation and perfusion scintigraphy was normally performed on the same day with the ventilation scan 3-4 hours before the perfusion scan. However, occasionally the perfusion scan was the first investigation to be performed. If this was abnormal the ventilation scan was performed the following day. Ventilation and perfusion scintigraphy was performed using a Gamma Camera (General Electric) fitted with a general purpose collimator. Images were acquired using a 20% energy window positioned over the 140 keV energy peak and a 128 matrix. The six standard views obtained were right posterior oblique, posterior, left posterior oblique, right anterior oblique, anterior and left anterior oblique. For the ventilation scan, patients breathed in 40 Mbq ^{99m}Tc labelled diethylenetriamine pentacetic acid (DTPA) in an aerosol form produced using a CIS Ventics 11 kit. The six standard views were acquired for 400kcps or 360 s, whichever was attained first. For the perfusion scan, patients in the prone position were given an intravenous injection of 80 Mbq ^{99m}Tc labelled macroaggregated albumin (MAA). The six standard views were acquired for 500kcps or 360s, whichever was attained first.

On completion of the study the radionuclide scans were classified into three groups (normal, high probability of pulmonary thromboembolism, and a third group including patients with a low or intermediate probability of pulmonary thromboembolism) by a consultant radiologist, blinded to the values of natriuretic peptides, based on the findings of the PIOPED study (The PIOPED Investigators, 1990, Gottschalk A et al, 1993).

Natriuretic peptide assays

Venous blood was taken from patients in the prone position at the time of the perfusion scan, prior to injection of Tc labelled MAA. Samples were collected and assayed as previously described. The coefficients of variability for each assay were: ANP interassay = 11.8%, intra-assay = 12.6%; BNP interassay = 14.8%, intra-assay = 9.9%; N-ANP interassay = 11.7%, intra-assay = 7.7%. Extraction efficiency for each assay was: ANP = 80%, BNP = 86% and N-ANP = 78%.

Data analysis

The individual data points for levels of natriuretic peptides in patients with normal, high probability and low/intermediate probability scans are represented in dot plots. Comparisons between these groups was made using the non-parametric Kruskal-Wallis test. Comparisons between levels of natriuretic peptides in patients with high probability scans who were alive on discharge or died during their hospital admission were made using the non-parametric Mann Witney test. A probability value of $p < 0.05$ (two tailed) was considered to be statistically significant. Values are quoted as median and interquartile ranges.

13.4 RESULTS

The study group consisted of 114 patients. Of the 114 ventilation perfusion scans performed 20 were reported as normal, 77 as representing low or intermediate probability of pulmonary embolism and 17 representing a high probability of pulmonary thromboembolism. Of the patients with a high probability of pulmonary thromboembolism 3 died of PTE and 14 were discharged home. On review of the clinical case notes all 17 patients with high probability scans were anticoagulated and had a clinical diagnosis of PTE made on the basis of clinical features and other investigations, including the result of the VQ scan. In contrast none of the patients with a normal VQ scan had a final clinical diagnosis of PTE.

Values are quoted for the median and interquartile ranges. There were statistically significant differences between groups for levels of a) BNP ($p<0.001$): N=6.7pmol/l (5.6-11.9), L/I=12.5pmol/l (6.7-28.2) and H=18.5pmol/l (12.6-74.6); b) ANP ($p<0.005$): N=12.6pmol/l (7.1-16.0), L/I=19.51pmol/l (12.5-28.2) and H=19.1pmol/l (15.7-31.7) and c) N-ANP ($p<0.05$): N=177 pmol/l (119-200), L/I=302 pmol/l (152-576) and H=322 pmol/l (223-563), (Figure 13.1). In addition levels of BNP and ANP were significantly ($p<0.05$) higher in patients with high probability scans who died of PTE than in those who survived; BNP: 91.6 pmol/l (77.5-336.2) vs 14.4 pmol/l (11.9-27.4) and ANP: 32.5 pmol/l (21.7-105.5) vs 17.6 pmol/l (15.2-19.3), and there was a non-significant trend towards a difference in levels of N-ANP ($p=0.07$) between these two groups (Figure 13.2).

TABLES

Table 13.1 Baseline characteristics of 114 participants with suspected PTE undergoing V/Q scanning.

	Probability of PE as defined by V/Q scan result		
Characteristic	Normal	Low/Intermediate	High
Number	20	77	17
Age (years)	46±18	64±15	69±11
Male (%)	25	39	65
Impaired LV function	1	6	3
PPH	0	2	1
Creatinine>120 mmol/l	2	14	4
COPD	0	18	1

Age is expressed as mean±standard deviation. PPH=primary pulmonary hypertension;
COPD=chronic obstructive pulmonary disease.

FIGURE LEGEND

Figure 13.1 Levels of natriuretic peptides in patients with suspected PTE.

The individual data points for levels of natriuretic peptides atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP) and N-terminal proatrial natriuretic peptide (N-ANP) in patients with normal (N), high probability (H) and low/intermediate probability scans (L/I) are represented in dot plots.

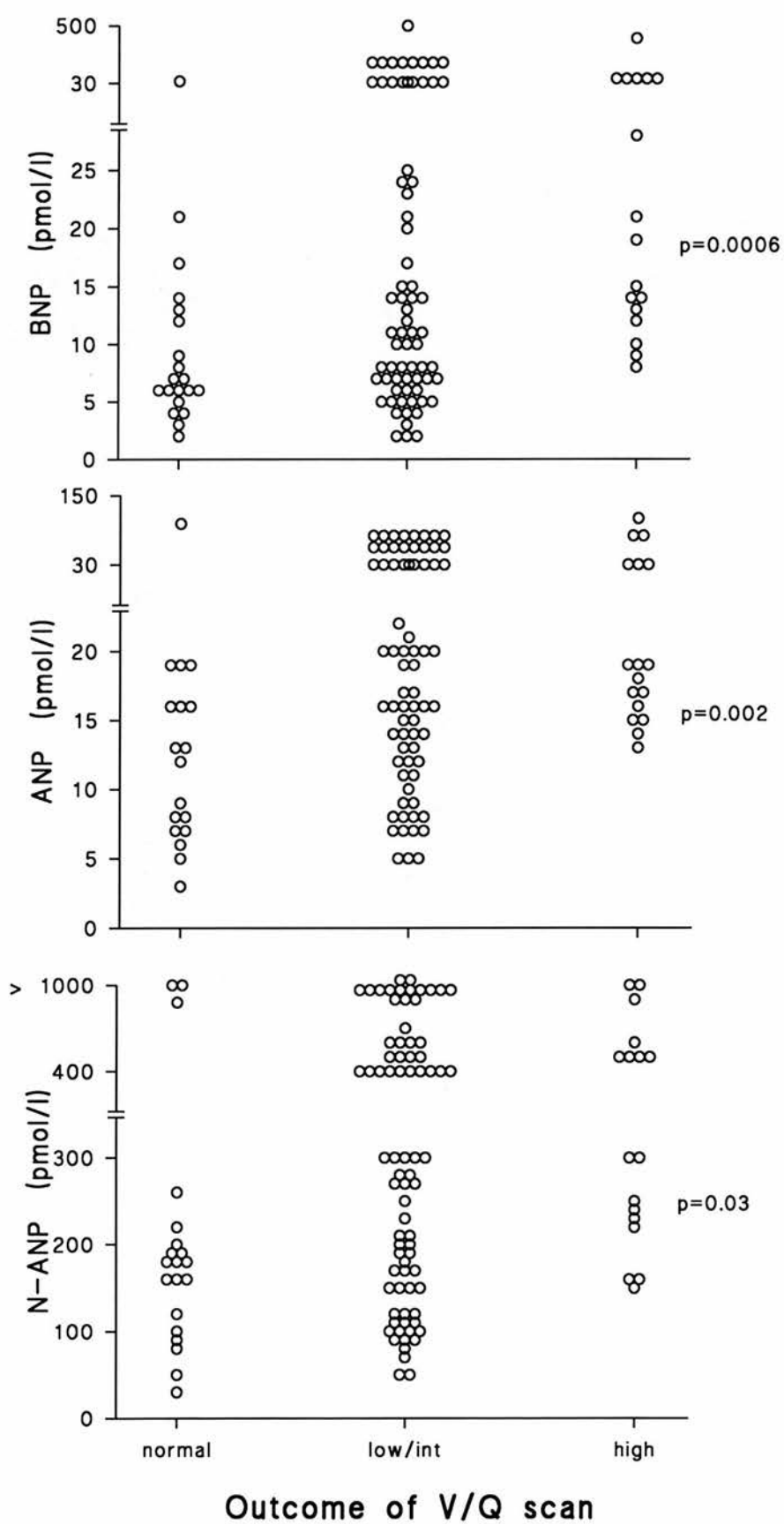
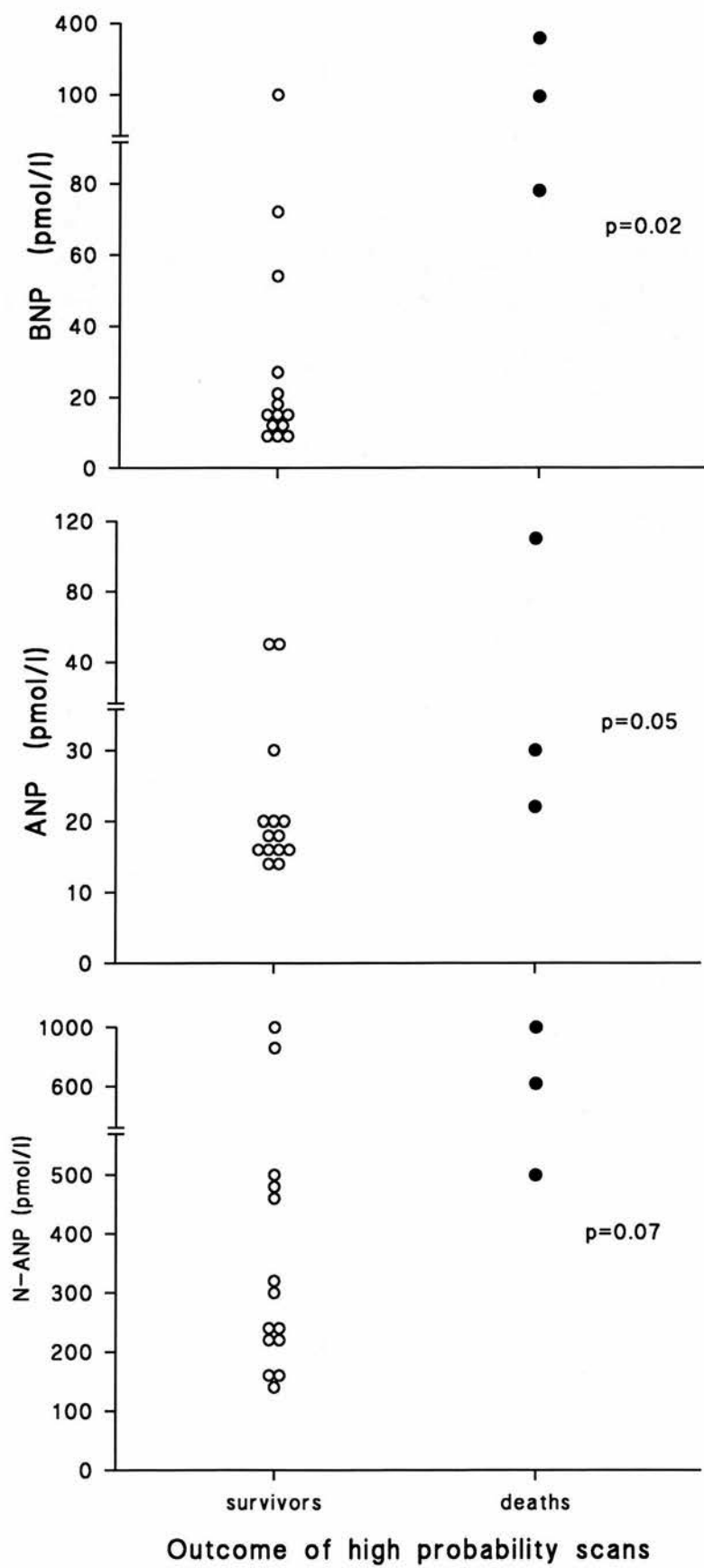


FIGURE LEGEND

Figure 13.2 Levels of natriuretic peptides in patients surviving or dying of PTE.

Comparisons between levels of natriuretic peptides atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP) and N-terminal proatrial natriuretic peptide (N-ANP) in patients with high probability scans who were alive on discharge or died during their hospital admission



13.5 DISCUSSION

This study is the first to report elevated levels of natriuretic peptides in patients with PTE. In addition although our numbers were small, patients with high probability scans who died had significantly elevated levels of natriuretic peptides compared to survivors. Perhaps suggesting that measuring levels of natriuretic peptides may have a role in identifying patients with PTE at high risk of sudden death.

Natriuretic peptides are thought to be released as a consequence of a stretch coupling mechanism and are elevated in a number of conditions characterised by elevations in left and right atrial and ventricular pressures such as left ventricular failure (McDonagh TA et al, 1998, Davidson NC et al, 1996), hypoxaemic cor pulmonale (Lang CC et al, 1992) and primary pulmonary hypertension (Morice AH et al, 1990). It is also known that acute hypoxia is a potent stimulus to ANP but not BNP (Cargill RI et al, 1996) secretion and the hypoxaemia seen in patients with pulmonary embolism may have contributed to elevated levels. We have also shown that levels of the inactive N-terminal portion of the 126 amino acid prohormone of ANP, N-ANP which is thought to reflect cardiac pressures over a more prolonged time period than ANP and BNP is elevated (Buckley MG et al, 1990). The natriuretic peptides have been shown to exert beneficial haemodynamic and endocrine effects by causing vasodilatation and natriuresis as well as antagonising the effects of the renin angiotensin system (Richards AM et al, 1988, Cargill RI et al, 1995). In pulmonary thromboembolism elevated levels of ANP and BNP are likely to reflect the cardiopulmonary consequences of PTE and may exert beneficial effects by causing pulmonary vasodilatation and reducing right ventricular afterload (Cargill RI et al, 1995). Elevated levels of natriuretic peptides, which are known to promote a natriuresis, offer an alternative explanation for the observation that patients with PTE may present with collapse in the lavatory. It is possible that rather than the art of

defaecation dislodging a clot (Kollef MH et al, 1991) that elevated levels of natriuretic peptides promote an intense desire to micturate.

A further interesting finding of this study was the significant difference in levels of natriuretic peptides in patients who had high probability scans who died and those who survived. Indeed levels of BNP were 15-100 times greater in patients with high probability scans who died than our laboratory's upper limit of normal (Davidson NC et al, 1996), suggesting a huge increase in right ventricular afterload. Perhaps measurement of levels of these peptides in conjunction with a specific diagnostic test may help to identify a group of patients at high risk who may benefit from more intensive early intervention.

What are the possible clinical applications of our results? The advent of new technology means that samples can be measured individually if required using a simple rapid assay (Murdoch D et al, 1997). As an adjunct to diagnosis BNP levels could easily be measured with rapid results providing information on the cardiopulmonary sequelae of pulmonary embolism without the need for invasive monitoring or specialised echocardiographic skills. In combination with either VQ scanning or helical computerised tomogram scanning of the thorax it may be possible to receive information regarding diagnosis and the degree of haemodynamic compromise. This in turn may help to select a group of patients at "high risk" who could then be managed in a high dependency unit or entered into trials to evaluate the role of thrombolysis in a more systematic manner than has been done previously. Measuring natriuretic peptide levels would also help us to identify patients with other conditions such as left ventricular failure (McDonagh TA et al, 1998, Davidson NC et al, 1996) and cor pulmonale (Lang CC et al, 1992) who have an increased mortality as a consequence of PTE (Carson JL et al, 1992), where elevated levels reflect the cardiopulmonary sequelae of both pulmonary embolism and the underlying disease process. Levels of

these peptides could also be used to follow up patients diagnosed as having had a pulmonary embolism as an indicator of clot resolution.

This paper reports for the first time elevated levels of natriuretic peptides in patients with PTE. Further work is required to evaluate more fully the potential diagnostic role of this peptide family, and in particular, whether measuring levels of natriuretic peptides may prove to be useful in risk stratification and identification of patients with PTE most likely to benefit from thrombolysis.

CONCLUSION AND OUTLOOK

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The work of this thesis has encompassed a series of studies in both normal man and patients with secondary pulmonary hypertension. The findings of these studies, including the limitations of both methodology and the pitfalls of extrapolation are discussed in Chapters 3 to 13. To avoid repetition the following synopsis refers to the core findings of these studies with particular reference to potential further research.

The first aim was to elucidate more fully the response of the cardiopulmonary system in normal man to hypoxaemia and hypercapnia. To help understand the importance of these physiological stimuli in health and thus give us clues to the cardiopulmonary sequelae of disease states characterised by these abnormalities of gaseous exchange. In particular we have examined the acute hypoxic vasoconstrictor response in normal man and the effects of hormonal manipulations of this response in health and disease.

The unassisted ascent of Mount Everest is testament to human endurance as well as a reflection of the ability of the heart to resist the effects of hypoxaemia. In Chapter 3 we demonstrated, using pulsed wave Doppler echocardiography and phonocardiography, that left ventricular systolic function and inotropic state are not affected by severe hypoxaemia. This is in contrast to the effects of myocardial ischaemia, such as occurs in myocardial infarction, and is consistent with the experimental findings that it is the accumulation of anaerobic metabolites that impairs myocardial contractility (Kihara Y et al, 1989). Acute hypoxaemia has been reported as increasing, decreasing or having no effect on cardiac output in humans. Our

findings of a dose dependent increase in cardiac output during hypoxaemia due to chronotropic effects rather than increases in stroke volume are consistent with the work of Phillips et al (Phillips et al, 1988). Indeed this physiological response to hypoxaemia makes adaptive sense by compensating for decreased oxygen saturation by increasing oxygen delivery. The apparent resistance of the inotropic function of the human myocardium to hypoxaemia is reflected in the well preserved systolic function of patients with cor pulmonale complicating COPD.

More recently diastolic function has emerged as an important determinant of overall cardiovascular performance (Clarkson PBM et al, 1994). Impaired relaxation of the myocardium occurs in a number of disease states including cor pulmonale secondary to COPD where at least right ventricular function is impaired.(Marangoni S et al, 1992). We examined whether functional abnormalities such as acute hypoxaemia could impair diastolic filling, in light of in vitro findings that acute hypoxaemia results in abnormalities of intracellular calcium transport. Using pulsed-wave Doppler echocardiography and phonocardiography we were able to demonstrate abnormalities of both left and right ventricular diastolic filling. Although the abnormalities of right ventricular diastolic function were of a similar magnitude to those seen in cor pulmonale complicating COPD (Marangoni S et al, 1992) the effects on left ventricular filling were less marked and more difficult to place in a clinical context. These findings are consistent with in vitro work and suggest that hypoxaemia may be an important aetiological factor in the development of diastolic filling abnormalities in COPD.

In Chapter 4 we have demonstrated for the first time in normal man that hypercapnia is capable of causing pulmonary vasoconstriction. We have shown that it is a weak pulmonary vasoconstrictor when compared with hypoxaemia. In humans hypercapnia may function as an intrinsic mechanism diverting blood from under ventilated areas of the lung in an effort to maintain ventilation perfusion matching in much the same way as hypoxaemia.

We have also examined the effects of a number of stimuli that have been suggested as important factors in arrhythmogenesis in respiratory disease using a novel marker of myocardial repolarisation, QT dispersion. This marker has been shown to be a powerful index for the propensity to develop life threatening arrhythmias (Barr CS et al, 1994). We have shown that both abnormalities of gaseous exchange and high doses of the potent β -agonist fenoterol increase QTc dispersion. This may be important in the aetiology of arrhythmias in acute severe asthma where β -agonists and abnormalities of gaseous exchange co-exist. Further work needs to be performed to see if this index predicts arrhythmia risk in patients with COPD.

The effects of hypoxaemia on the pulmonary vasculature have been studied extensively since Von Euler and Liljestrand described the phenomenon of hypoxic pulmonary vasoconstriction in the cat in 1946. Since then there have been great strides in understanding some of the mechanisms of this response although the site of the "oxygen sensor" and the exact way in which hypoxia results in pulmonary vasoconstriction is unknown (NF Voelkel, 1996). We have examined the role of ANG II in modulating this response in man in health and disease based on a series of

observations suggesting that ANG II may play an important role. Firstly ANG II is a potent pulmonary pressor in man (Segel N et al, 1960), levels of this peptide are elevated in patients with hypoxaemic cor pulmonale (Peacock AJ et al, 1992) and ACE inhibitors have been shown to attenuate the development of pulmonary hypertension in chronically hypoxic rats (Zakheim RM et al, 1975). There is, however, some concern that certain animal models may not be a good model for pulmonary hypertension in man, where for example the reaction of the pulmonary vasculature of the rat is very different to that of the human lung. (D Heath et al, 1996). Our studies were therefore limited to man and have been aided by the advent of non-invasive methodology which has removed the ethical constraints of performing some of these studies. In a series of studies in normal man we have demonstrated that ANG II is capable of modulating the acute HPV response using infusions of the angiotensin II receptor blocker saralasin and the orally active type 1 ANG II blocker losartan. This work was then extended to patients with hypoxaemic cor pulmonale where we demonstrated both modest beneficial pulmonary haemodynamic changes at rest in addition to beneficial endocrine changes. A number of small acute dosing studies have also been performed by other investigators studying pulmonary haemodynamic changes in patients treated with ACE inhibitors. These have been limited by small patient numbers and inconsistent inclusion criteria which may explain their variable effect on pulmonary haemodynamics. In our small study which shows only a modest pulmonary haemodynamic benefit we selected patients who had vasoreactive pulmonary circulations (based on changes in their MPAP in response to inspired oxygen in an analogous manner to asthmatics who are selected for bronchoreactivity prior to studying the effects of new bronchodilators). The clinical

significance of these results is unknown but the benefits from a pulmonary haemodynamic point of view in end stage disease, when put in the context of other studies, are likely to be modest and our current clinical practice is not to routinely use these drugs in the treatment of pulmonary hypertension complicating COPD.

Undoubtedly, if we are to try and definitively answer the question as to whether vasodilators have a role to play in managing secondary pulmonary hypertension complicating COPD we must ask ourselves whether it is likely to have benefits. Indeed there is some debate as to the importance of pulmonary hypertension in terms of influencing morbidity and mortality in COPD. Our feeling is that it is important. This is reflected in part by the finding that the level of MPAP is a good indicator of prognosis in COPD. This has been shown in large studies by (Weitzenblum E et al, 1981) and Bishop et al (Bishop JM et al, 1984) who found that the level of pulmonary hypertension was a predictor of mortality. Although the increases in MPAP at rest are modest when compared with primary pulmonary hypertension it is well recognised that these pressures can increase 20-30 mmHg during exercise, sleep and respiratory failure resulting in increased right ventricular afterload and right heart failure. Indeed activities such as climbing stairs or walking can result in significant increases in pulmonary artery pressure which are perceived by the patient as breathlessness suggesting that treatment that can attenuate this increase in pressure may improve morbidity. It is recognised that oxygen therapy as well as improving mortality can reverse or attenuate the development of pulmonary hypertension (Weitzenblum E et al, 1985, Report of the Medical Research Council, 1981) and that there is an association between a fall in MPAP and survival. It seems logical,

therefore, not only to treat the underlying lung disease but also its pathophysiological sequelae. To achieve this end we need to conduct large long term follow up studies with concrete end points. In addition to using a vasodilator, that we feel is likely to have beneficial effects on for example, haemodynamics and vascular remodelling. In our eyes the “jury remains out” until such studies are conducted.

The development of pulmonary hypertension in hypoxic lung disease in the setting of COPD is complex and reflects an interplay between acute hypoxic pulmonary vasoconstriction, pulmonary vascular remodelling, ablation of the pulmonary vascular bed and more recently the recognition that abnormalities of endothelial function are likely to play an important role. Pulmonary vasoconstriction may result from endothelial dysfunction with an imbalance of vasodilators such as nitric oxide and prostacyclin and vasoconstrictors such as ANG II and ET-1. Indeed endothelium dependant relaxation is reduced in chronically hypoxic rats and in man in end stage chronic hypoxic lung disease endothelium dependant relaxation mediated via nitric oxide is impaired (Dinh-Xuan AT et al, 1991). In man evidence suggests that basal NO production is important in maintaining systemic vascular tone (Vallance P et al, 1989, Haynes WG et al, 1993) however, there is some debate as to the role of NO in maintaining the low pressure pulmonary circulation in man where conflicting results exist suggesting the possibility of species specificity. In chapter 12 we have shown for the first time in a placebo controlled manner that basal nitric oxide generation plays an important role in maintaining both systemic and pulmonary vascular tone as well as cardiac output in normal man. These results concur with the findings of Dinx-Yuan et al suggesting that compromise of the normal constitutive mechanisms of NO

production may have direct pathophysiological consequences. The delivery of NO to the lung in the treatment of hypoxic lung disease complicating COPD holds exciting prospects.

Following identification of endothelial derived relaxing factors attention was again turned to identifying potent vasoconstrictors. In 1988 ET-1 was isolated as the most potent vasoconstrictor substance known to man. Growing evidence suggests that ET-1 plays an important role in modulating the vascular response to hypoxaemia. Although levels of this peptide were known to be elevated in primary pulmonary hypertension (Stewart DJ et al, 1991) the effects of hypoxaemia on ET-1 release at sea level and the effects of ET-1 on the pulmonary vascular bed in normal man were not known. We have demonstrated for the first time that acute hypoxaemia results in ET-1 release in normal man, that levels of this peptide are elevated in cor pulmonale complicating COPD and that infusions of this peptide are capable of causing pulmonary vasoconstriction in a dose dependant manner in the integrated physiological system of man. It is possible that the potent vasoactive properties of ET-1 may be responsible for some of the circulatory abnormalities seen during hypoxaemia. In cor pulmonale the elevated levels of ET-1 may have adverse effects by causing pulmonary vasoconstriction as well as affecting pulmonary vascular remodelling. Additionally, we have also shown that ET-1 has adverse dose-dependant effects on both systolic and diastolic left and right ventricular function. These novel findings may be important in considering the pathophysiological role of ET-1 in both cardiovascular and cardiopulmonary disease. Whether ET-1 acts as a mediator of acute HPV is unknown. Support or otherwise for this hypothesis may be drawn from

studies using endothelin receptor blockers which have been shown to attenuate hypoxic pulmonary vasoconstriction (Oparil S et al, 1995) and prevent pulmonary hypertension following chronic hypoxia in the rat (Eddahibi S et al, 1995). Work needs to be performed using a human model to see if ET-1 receptor blockade attenuates acute HPV and in patients with hypoxaemic lung disease to see if ET-1 receptor blockade is of value in the treatment and or prevention of the cardiopulmonary consequences of chronic hypoxaemia.

In the final chapter of this thesis we have examined the effects of pulmonary thromboembolism on the natriuretic peptide system. In recent times there has been much interest in the diagnostic potential of these peptides in left ventricular failure where they have been shown to be sensitive markers of left ventricular systolic dysfunction. This study is the first to our knowledge to report elevated levels of natriuretic peptides in PTE in a group of 114 patients in whom the diagnosis was suspected. Although we have no data from this study to support our observations we believe that elevated levels of ANP, BNP and N-ANP are likely to reflect the well documented cardiopulmonary consequences of PTE. Although the numbers were small an interesting finding of this study was the significant difference in levels of natriuretic peptides in patients who died of PTE compared to survivors. Indeed for BNP, levels were 15-100 times greater in patients who died of PTE compared to our laboratories upper limit of normal suggesting a huge increase in right ventricular overload. The advent of new technology allows individual measurement of samples with rapid results (Murdoch D et al, 1997) providing us with the potential to stratify patients (who have also had a diagnostic test) into those at low and high risk of death

without the need for invasive monitoring or specialised echocardiographic skills. This in turn may help us to select patients at “high risk” who could be managed in a high dependency unit or considered for thrombolysis.

To confirm the findings of this pilot study we are in the process of organising a 2 stage trial using spiral CT scanning as a diagnostic tool in addition to performing echocardiography and measuring levels of natriuretic peptides of PTE in all patients with suspected PTE. In addition we will perform right heart catheterisation to measure pulmonary haemodynamic changes and indices of right ventricular performance in a cohort of this group. If the findings of the first part of his study confirm the findings of our pilot study we then plan to use natriuretic peptides as an indicator of “high risk” in the acute situation and randomise high risk patients to receive thrombolysis or heparin.

In conclusion. The pulmonary vascular bed is more than just a passive conduit for the transport of blood from the right to the left side of the heart. The appreciation of the role of vasoactive mediators, endothelial function and vascular remodelling hold the key to unravelling some of the mysteries of the pulmonary vascular bed.

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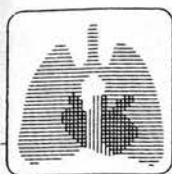
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APPENDIX

The following papers resulted from this MD thesis. Where photocopies are shown the publishers formal permission was obtained as well as permission from joint authors.



clinical investigations

Left Ventricular Systolic Performance During Acute Hypoxemia*

Robert I. Cargill, MB; David G. Kiely, MB; and Brian J. Lipworth MD

Study objective: Although some of the cardiovascular responses to hypoxemia are well described, effects on myocardial contractility have not been defined. Such effects are readily assessed by noninvasive techniques and we have therefore evaluated Doppler-phonocardiographic parameters of systolic left ventricular contractility in normal humans rendered hypoxemic.

Design: Eight healthy male volunteers were studied. Parameters were measured after resting to achieve baseline haemodynamics, after 20 min moderate hypoxemia (SaO_2 85 to 90%), and after a further 20 min of severe hypoxemia (SaO_2 75 to 80%). Hypoxemia was induced by breathing a variable N_2/O_2 mixture.

Measurements: Pulsed-wave Doppler analysis of ascending aortic blood flow was combined with phonocardiography to measure indices of systolic left ventricular function at baseline and at the end of each period of hypoxemia.

Results: There was a significant, dose-related increase in cardiac output in response to hypoxemia, from 5.5 ± 0.26 L/min at baseline to 6.1 ± 0.08 L/min during moderate hypoxemia and to 7.0 ± 0.23 L/min during severe hypoxemia. Likewise, heart rate increased significantly in dose-related fashion although stroke volume was not affected by either level of hypoxemia. Hypoxemia had no significant effects on systolic or diastolic blood pressures, but caused a significant reduction in systemic vascular resistance. Aortic peak

and mean acceleration and acceleration time were not affected by moderate or severe hypoxemia. Although the systolic time intervals measured shortened significantly during severe hypoxemia, these were no longer significant when appropriate corrections were made for heart rate.

Conclusions: Although cardiac output increases during hypoxemia, this is due to increases in heart rate but not to any effect on stroke volume. Parameters of left ventricular systolic function and myocardial inotropic state were also not affected by severe hypoxemia. Systolic left ventricular function and myocardial contractility are thus well preserved in normal humans during hypoxemia. (CHEST 1995; 108:899-902)

Acc_{mean}=mean aortic acceleration; Acc_{peak}=peak aortic acceleration; AT=aortic acceleration time; CI=confidence interval; CO=cardiac output; CSA=aortic cross-sectional area; ET=left ventricular ejection time; HR=heart rate; MAP=mean arterial pressure; PEP=pre-ejection period; QS₂=time interval from ECG Q wave to phonocardiogram aortic closure sound; SaO₂=oxygen saturation; SV=stroke volume; SVI=aortic systolic velocity integral; SVR=systemic vascular resistance

Key words: Doppler; hemodynamics; hypoxemia; systolic function

Acute hypoxemia commonly occurs in humans as a result of cardiorespiratory disease or adverse environmental conditions. The cardiovascular responses to the stress of hypoxemia have therefore been of great

interest. It is clear that acute hypoxemia induces pulmonary hypertension due to hypoxic pulmonary vasoconstriction,¹ and increases heart rate (HR) and cardiac output (CO)² without affecting systemic blood pressure.^{2,3} To our knowledge, there is no information

available regarding the effects of hypoxemia on intrinsic myocardial contractility during systole. Such effects may well be important in the assessment of cardiovascular performance in subjects with fluctuating levels of oxygenation.

The noninvasive assessment of systolic ventricular function has become particularly important with the advent of effective therapies for systolic heart failure.⁴ Although quantification of ejection fraction appears a suitable measure of systolic function, this is subject to other haemodynamic influences⁵ and does not directly assess intrinsic myocardial contractility. This latter component, however, can be assessed by a number of other noninvasive methods, including echocardiography and phonocardiography. Echo-Doppler assess-

For editorial comment see page 889

From the Department of Clinical Pharmacology, Ninewells Hospital and Medical School, University of Dundee, Scotland. Manuscript received January 26, 1995; revision accepted March 24.

Table 1—Systemic Hemodynamic Parameters*

	Baseline	Hypoxemia	
		Moderate	Severe
HR, (beats/min)	60±2.7	65±2.8 [†]	71±4.3 [‡]
SV, mL	102±7.5	102±4.7	104±4.9
CO, L/min	5.5±0.26	6.1±0.08 [†]	7.0±0.23 [‡]
Systolic BP, mm Hg	120±7.5	125±6.8	125±6.9
Diastolic BP, mm Hg	65±3.8	70±3.7	67±4.0
SVR, dyne·s·cm ⁻⁵	1,224±80	1,085±36 [†]	979±59 [†]

*Absolute values (mean±SEM) of heart rate (HR), stroke volume (SV), cardiac output (CO), systolic blood pressure, diastolic blood pressure, and systemic vascular resistance (SVR) at baseline and during moderate and severe hypoxemia.

[†]Significantly different from baseline.

[‡]Significantly different from moderate hypoxemia.

ment of aortic flow acceleration parameters has been used to study changes in overall ventricular performance and compares favorably with invasive measurements.⁶ Other systolic time intervals, derived in conjunction with phonocardiography, have also been proposed to reflect the inotropic state of the left ventricle⁷ and have been used extensively to monitor drug-induced changes in myocardial contractility.^{8,9}

We have therefore combined these two methods to assess for the first time the effects of moderate and severe acute hypoxemia on parameters of systolic myocardial performance in humans.

METHODS

Study Design

Eight normal male volunteers, age (mean±SEM) 29.3±2.9 years, were evaluated after giving informed written consent to the study protocol, previously approved by the Tayside Committee for Medical Research Ethics. All subjects had normal clinical history and examination results, biochemical and hemotologic screening, and 12-lead electrocardiogram.

All subjects attended the clinical laboratory at the same time of day and remained in a supine position throughout the study. Once stable resting hemodynamic parameters were obtained, subjects were rendered hypoxemic by breathing a variable nitrogen/oxygen mixture. Gases were mixed in a 25-L Douglas bag from separate cylinders fitted with variable flow valves. Subjects breathed from this reservoir through a mouthpiece connected by a series of one-way valves, while wearing an occlusive nose clip. Relative gas concentrations were adjusted to achieve moderate hypoxemia with pulse oximeter (CSI 503; Criticare Systems Inc; Waukesha, Wis) monitoring of oxygen saturation (SaO₂) between 85 and 90% for 20 min followed by 20 min of severe hypoxemia with SaO₂ between 75 and 80%. Parameters were recorded at baseline and at the end of each 20-min period of hypoxemia.

Measurements

Systemic Hemodynamic Variables: Heart rate was recorded by electrocardiograph and rate was averaged over 60 s. Blood pressure was monitored by semiautomatic sphygmomanometer (Vital Signs Monitor; Critikon; Tampa, Fla). To measure CO, aortic cross-sectional area (CSA) was first measured by M-mode echocardiography (Vingmed SD50; Vingmed Sound, Horten, Norway) from the left parasternal view at the level of the aortic root. The aortic systolic

flow velocity integral (SVI) was then measured by computer-assisted determination from the pulsed-wave Doppler profile of aortic blood flow from the suprasternal notch allowing stroke volume (SV=SVI×aortic cross-sectional area [CSA]) and CO to be calculated on line. Systemic vascular resistance (SVR) was calculated as (mean arterial pressure/CO)×80 dyne·s·cm⁻⁵.

Systolic Flow Parameters: Simultaneous lead II ECG, phonocardiogram (Siemens AG; Munich, Germany), and pulsed-wave Doppler ascending aortic blood flow (Vingmed SD50) were recorded. Doppler flow profiles were recorded with a nonimaging 2.0-MHz transducer with depth adjusted to give maximal velocity. All measurements were recorded onto videotape with display sweep speed at 100 mm/s and analyzed in triplicate at the end of the study. From these recordings, the following variables were measured: Aortic peak acceleration (Acc_{peak}), aortic mean acceleration (Acc_{mean}), aortic acceleration time (AT) as time in milliseconds from onset to peak aortic flow, left ventricular ejection time (ET) as time in milliseconds from onset to end of systolic aortic flow, interval from ECG Q wave to second heart sound on phonocardiogram (QS₂) and pre-ejection period (PEP) as time in milliseconds from ECG Q wave to onset of systolic aortic flow. QS₂, ET, and PEP were also corrected for changes in HR according to standard criteria,¹⁰ and are denoted QS_{2c}, ET_c, and PEP_c. We have previously shown the coefficients of variability for measurement by these methods of SVI to be 1.2%, Acc_{mean} to be 12.5%, AT to be 6.4%, and ET to be 1.6%.¹¹

Data Analysis

Comparisons were made by multifactorial analysis of variance and where this was significant, Duncan's multiple-range testing was used to determine where significant differences lay.¹² A probability value of *p*<0.05 (two-tailed) was considered to be statistically significant.

RESULTS

Systemic Hemodynamic Changes

Moderate hypoxemia significantly increased CO by 0.7 L/min (95% confidence interval [CI], 0.1 to 1.3) and HR by 4.5 beats/min (95% CI, 0.7 to 9.7) from baseline. Severe hypoxemia caused further increases in CO, 1.5 L/min (95% CI, 0.9 to 2.1), and HR, 10.5 beats/min (95% CI, 5.3 to 15.7) (Table 1). The SV, however, was not affected by hypoxemia, while systolic and diastolic blood pressure were unchanged during moderate or severe hypoxemia (Table 1). SVR decreased significantly during moderate hypoxemia (−138 dyne·s·cm⁻⁵ from baseline; 95% CI, 2 to −274) and during mild hypoxemia (−245 dyne·s·cm⁻⁵ from baseline; 95% CI, 109 to 381) (Table 1).

Systolic Flow Parameters

Acc_{peak} was similar at baseline (23.8±1.5 ms⁻¹) and after moderate (22.1±1.0 ms⁻¹) and severe hypoxemia (21.4±2.5 ms⁻¹). Similarly, Acc_{mean} did not change from baseline (10.9±1.0 ms⁻¹) during either moderate (10.4±1.0 ms⁻¹) or severe hypoxemia (12.0±1.8 ms⁻¹). AT was also unchanged from baseline (76±6.7 ms) during either moderate (75±6.5 ms) or severe hypoxemia (73±6.7 ms).

Although similar at baseline (411±8 ms) and during moderate hypoxemia (410±8 ms), QS₂ was signifi-

significantly shortened during severe hypoxemia (385 ± 11 ms). Likewise ET was similar at baseline (293 ± 9 ms) and during moderate hypoxemia (289 ± 10 ms) but shortened significantly during severe hypoxemia (277 ± 10 ms). PEP was also significantly shorter during severe hypoxemia (109 ± 8 ms) than at baseline (118 ± 9 ms) or during moderate hypoxemia (121 ± 8 ms). However, following correction of these parameters for HR changes, QS_{2c}, ET_c, or PEP_c were not significantly affected by either level of hypoxemia (Fig 1).

DISCUSSION

We have shown that while causing significant changes in haemodynamic status, hypoxemia does not impair left ventricular myocardial contractility in normal humans. It is therefore appropriate to consider these hemodynamic effects separately from indices of myocardial contractility.

These observations are in agreement with the work of Phillips et al² who also noted using similar methods, that increases in CO during hypoxemia were the result of positive chronotropic effects rather than any effect on SV. The pulsed-wave Doppler techniques we have employed, however, are more accurate and reproducible,¹³ comparing favorably with invasive measurement of CO.^{14,15} Unlike Phillips et al,² we did not control PaCO₂ in our subjects. However, in view of the concordant findings, it is unlikely that this has a bearing on CO over the SaO₂ range studied.

We also calculated SVR which fell significantly during hypoxemia. The consequent decrease in left ventricular afterload by itself might therefore be expected to cause an increase in SV. Since SV did not change during hypoxemia, this may have been limited by the increased right ventricular afterload secondary to hypoxic pulmonary vasoconstriction. The constant SV we observed would therefore make it less likely that mechanical factors in accordance with Starling's law (ie, left ventricular distention) could have affected systolic left ventricular contractility during hypoxemia.

Of the Doppler aortic acceleration indices, both mean and peak acceleration have been used as markers of left ventricular contractility,¹⁶ although Acc_{peak} appears to be relatively less influenced by changes in loading conditions.¹⁷ Furthermore, although Acc_{peak} declines with increasing HR during pacing,¹⁸ the effects across the HR range of our study are small and consistent with the nonsignificant decrease observed. Thus, hypoxemia has no significant effects on left ventricular contractility as measured by either peak or mean aortic acceleration.

The systolic time intervals are easily measured and repeatable markers of the inotropic state of the left ventricle,⁷ which are in some cases more sensitive than echo-Doppler parameters.¹⁹ Their changes with heart

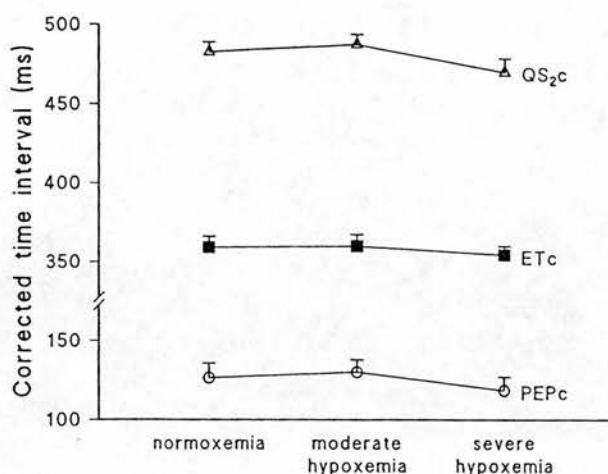


FIGURE 1. Systolic time intervals, corrected for changes in heart rate, showing QS_{2c}, left ventricular ejection time (ET_c), and pre-ejection period (PEP_c) at baseline and during moderate and severe hypoxemia.

rate are well established and corrections appropriate to the study population are easily applied.¹⁰ As measures of the inotropic state of the myocardium, the systolic time intervals are prolonged if contractility is impaired with the QS₂ interval being relatively unaffected by changes in loading conditions.⁷ We found that hypoxemia did not affect heart rate corrected QS₂, as has been found previously by Bremner et al,²⁰ or any of the other systolic time intervals measured. We can therefore be confident that hypoxemia did not significantly alter left ventricular myocardial contractility.

It would appear therefore that systolic contractility of normal human myocardium is relatively resistant to the effects of hypoxemia. This is in contrast to the effects of myocardial ischemia in which, in addition to myocyte hypoxia, limitation of blood flow might also allow accumulation of anaerobic metabolites that impair contractility.²¹ These findings may well be important in determining the relevance of hypoxemia as a cause of abnormal cardiovascular performance in hypoxemic patients as well as in assessing hemodynamic responses to changes in oxygenation status.

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Adverse effects of hypoxaemia on diastolic filling in humans

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1. Abnormalities of myocardial relaxation may occur as a consequence of myocyte hypoxia. We have therefore examined the effects of hypoxaemia on right and left ventricular diastolic function in 10 healthy male subjects.

2. After resting to reach baseline haemodynamics, subjects were rendered hypoxaemic by breathing a variable nitrogen/oxygen mixture. Oxygen saturation (SaO_2) was maintained at 85–90% for 20 min and then at 75–80% for a further 20 min. Haemodynamic and diastolic filling parameters were measured non-invasively at baseline and at the end of each period of hypoxaemia.

3. Diastolic filling of both ventricles was significantly impaired by hypoxaemia. In comparison with baseline, left ventricular isovolumic relaxation time and transmitral E-wave deceleration time corrected for heart rate were significantly prolonged at SaO_2 75–80%: mean difference in corrected relaxation time, 9.8 ms (95% confidence interval 1–19); mean difference in corrected deceleration time, 34 ms (95% confidence interval 11–56). Similarly, right ventricular isovolumic relaxation time and transtricuspid E-wave deceleration time were significantly prolonged at SaO_2 values of 75–80% compared with baseline: mean difference in relaxation time, 20.3 ms (95% confidence interval 3–38); mean difference in deceleration time, 33 ms (95% confidence interval 11–55).

4. During hypoxaemia there were dose-related increases in heart rate, cardiac output and mean pulmonary artery pressure, but no effects on mean arterial pressure.

5. Hypoxaemia significantly impairs relaxation of left and right ventricles in normal humans. These changes may reflect impairment of intracellular calcium transport secondary to the effects of myocyte hypoxia.

marker of cardiac dysfunction. Abnormal diastolic function precedes systolic function abnormalities in a number of disease states, including ischaemic heart disease [2], hypertrophic cardiomyopathy [3] and hypertensive left ventricular hypertrophy [4], and may contribute to the morbidity of these conditions.

Although most human studies of diastolic function have evaluated filling patterns of the left ventricle, the role of ventricular interaction during diastole [5, 6] might indicate the need to consider abnormalities of right and left ventricular filling together. In this respect, right as well as left ventricular filling is impaired in patients with ischaemic heart disease [7] and restrictive cardiomyopathy [8], while patients with pulmonary hypertension have paradoxical impairment of left ventricular filling [6].

In many of the above conditions, it is difficult to differentiate between structural cardiac changes (e.g. ventricular remodelling) and functional abnormalities related to changes in myocyte metabolism. Cellular hypoxia and the consequent abnormalities of intracellular calcium transport [9] may impair myocyte relaxation and hence may worsen overall diastolic function. These cellular events may be of relevance during myocardial ischaemia which leads to local hypoxia, or if hypoxaemia accompanies cardiopulmonary disease, as in cor pulmonale, in which at least right ventricular diastolic function is impaired [10].

To elucidate further the role of these functional abnormalities during diastole, we have studied the effects of hypoxaemia in normal humans. In this setting structural abnormalities can be excluded, allowing us to study *in vivo* the functional effects of hypoxaemia on left and right ventricular diastolic filling in man.

INTRODUCTION

Ventricular diastolic function has emerged as an important determinant of overall cardiovascular performance [1] and is a particularly sensitive

METHODS

Subjects

Ten normal male subjects, age (mean \pm SEM) 28.0 ± 1.9 years, were evaluated. All subjects had no

Key words: diastolic function, Doppler analysis, hypoxaemia.

Abbreviations: CO, cardiac output; CSA, cross-sectional area; EDT, E-wave deceleration time; HR, heart rate; IVRT, isovolumic relaxation time; MAP, mean arterial pressure; MPAP, mean pulmonary arterial pressure; PAT, pulmonary flow acceleration time; SV, stroke volume; SVI, systolic flow velocity integral.

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abnormality on clinical history and examination, biochemical and haematological screening or 12-lead electrocardiogram. In addition, echocardiographic study was required to be normal with high-quality transmitral and transtricuspid Doppler flow profiles. Informed written consent to the study protocol, previously approved by the Tayside Committee for Medical Research Ethics, was obtained from each subject.

Study protocol

Subjects attended the clinical laboratory between 2 and 4 h after lunch and remained in a supine position, rolled slightly on to the left side. After resting for at least 40 min to obtain stable resting haemodynamic parameters, subjects were rendered hypoxaemic by breathing a variable mixture of nitrogen and oxygen. The hypoxic gas mixture was produced from separate cylinders of nitrogen and oxygen fitted with variable flow valves, mixed in a 25-l Douglas bag, from which subjects breathed through a mouth-piece connected by a series of one-way valves, while wearing an occlusive nose clip. Precise concentrations of each gas were adjusted to achieve an oxygen saturation (SaO_2) between 85% and 90% for 20 min followed by 20 min with SaO_2 between 75% and 80%. Haemodynamic variables and diastolic filling parameters were recorded at baseline and at the end of each period of hypoxaemia at steady state.

Measurements

Oxygen saturation. Arterial blood oxygen saturation was continuously monitored by transcutaneous pulse oximetry (CSI 503; Criticare Systems, Waukesha, WI, U.S.A.).

Haemodynamic variables. Heart rate (HR) was recorded on an electrocardiograph trace and an average rate over 1 min was obtained. To measure cardiac output (CO), aortic cross-sectional area (CSA) was first measured by M-mode echocardiography (Vingmed SD50; Vingmed Sound, Horten, Norway) from the left parasternal view at the level of the aortic root. The aortic systolic flow velocity integral (SVI) was then measured by on-line computer-assisted determination from the pulsed-wave Doppler profile of aortic blood flow from the suprasternal notch. On-line determination of stroke volume ($\text{SV} = \text{SVI} \times \text{CSA}$) and hence CO, as the product of SV and HR, was calculated.

Mean arterial pressure (MAP) was measured by semiautomatic sphygmomanometer (Vital Signs Monitor; Critikon, Tampa, FL, U.S.A.). Mean pulmonary artery pressure (MPAP) was calculated from measurement of pulmonary flow acceleration time (PAT) by pulsed-wave Doppler echocardiography (Vingmed SD50) from the left third/fourth intercostal space as previously described [11] using the regression equation $\text{MPAP} = 73 - (0.42 \times$

$\text{PAT})$ [12]. The mean of three consistent waveforms at each time point was used for the purpose of analysis for each Doppler-derived parameter. We have previously shown the short term coefficients of variation for measurement of PAT and SVI to be 1.7% and 1.2% respectively [11].

Diastolic filling parameters. From the apical window, pulsed-wave Doppler analysis of mitral and tricuspid diastolic flow (Vingmed SD50) was combined with simultaneous phonocardiogram recording with the microphone (Siemens, Munich, Germany) positioned over the second left intercostal space. Measurements were all made on-line during expiration and in triplicate, with a display sweep speed of 100 mm/s. Transmitral and transtricuspid flow was analysed after adjusting sample volume depth to yield maximal E-wave velocities with clearly defined flow velocity envelopes. Measurement of diastolic flow parameters from these signals has previously been shown to be highly reproducible and easily applicable in our own laboratory [11] and also by other workers [13]. Aortic and pulmonary components of the second heart sound were identified on the phonocardiogram trace by noting closure artefacts from superimposition of aortic and pulmonary Doppler flow profiles.

From diastolic transmitral and transtricuspid flow, maximal velocities of the early ($E_{v_{\max}}$) and atrial ($A_{v_{\max}}$) components of flow were measured, and the E/A ratio was calculated. In addition, the E-wave deceleration time (EDT) was calculated as the time in milliseconds from peak velocity to the end of the E-wave. The isovolumic relaxation time (IVRT) was calculated for the left ventricle as the time in milliseconds from the aortic component of the phonocardiogram second heart sound to the onset of diastolic transmitral flow, and for the right ventricle as the time from the pulmonary component of the phonocardiogram second heart sound to the onset of diastolic transtricuspid flow. In our hands, the short-term coefficients of variation for these measurements were as follows: mitral EDT, 2.4%; tricuspid EDT, 4.8%; left ventricular IVRT, 1.1%; and right ventricular IVRT, 12.2%. Both time intervals (EDT and IVRT) were also corrected for changes in heart rate induced by hypoxaemia by dividing by the square root of the simultaneous ECG R-R interval; $\text{EDTc} = \text{EDT} / \sqrt{\text{RR}}$; $\text{IVRTc} = \text{IVRT} / \sqrt{\text{RR}}$.

Data analysis

Comparisons were made by multifactorial analysis of variance and, where this was significant, Duncan's multiple-range testing was used to determine where significant differences lay [14]. A probability value of $P < 0.05$ (two-tailed) was considered to be statistically significant. Data are presented in the text and figures as means and SEM, and where a difference between means is quoted the 95% confidence interval for this difference is also given.

Table 1. Haemodynamic changes. Absolute values (means \pm SEM) of heart rate (HR), cardiac output (CO), mean arterial pressure (MAP) and mean pulmonary artery pressure (MPAP) at each level of oxygenation. *Significantly different from >95%. †Significantly different from 85–90%.

	SaO ₂		
	>95%	85–90%	75–80%
HR (beats/min)	62.1 \pm 2.9	67.9 \pm 3.5*	74.5 \pm 4.5*†
CO (l/min)	5.59 \pm 0.28	6.41 \pm 0.41*	7.22 \pm 0.42*†
MAP (mmHg)	85.0 \pm 3.4	87.2 \pm 3.3	88.1 \pm 3.6
MPAP (mmHg)	9.3 \pm 0.4	17.8 \pm 0.8*	27.6 \pm 1.0*†

RESULTS

Haemodynamic changes

Hypoxaemia caused significant dose-related increases in both HR and CO (Table 1). Hypoxic pulmonary vasoconstriction resulted in an increase in MPAP at both levels of hypoxaemia, while no significant effects on systemic MAP were observed (Table 1).

Diastolic function parameters

Transmitral E/A ratio decreased progressively in response to increasing levels of hypoxaemia (Fig. 1a) as a result of significant increases in A-wave velocity, whereas E-wave velocity was unchanged (Table 2). A similar pattern was observed for trans-tricuspid flow, reductions in the E/A ratio in response to hypoxaemia (Fig. 1a) being due to increases in Av_{max} rather than changes in Ev_{max} (Table 3).

Absolute levels of left ventricular IVRT were not significantly affected by hypoxaemia (Table 2) although, after correction for changes in HR, IVRTc was significantly prolonged from baseline when SaO₂ was 85–90% (mean difference 8.9 ms; 95% confidence interval 1–16) and when SaO₂ was 75–80% (mean difference 9.8 ms; 95% confidence interval 1–19) (Fig. 1b). In the right ventricle, IVRT was prolonged when SaO₂ was reduced to 75–80% but not at 85–90% (Table 3). In comparison with baseline, right ventricular IVRTc was prolonged by hypoxaemia at SaO₂ 75–80% (mean difference 20.3 ms; 95% confidence interval 3–38) but not at SaO₂ 85–90% (Fig. 1b).

Mitral EDT was only prolonged at SaO₂ 75–80% (Table 2). After correction for HR, EDTc was also significantly prolonged from baseline at SaO₂ 75–80% (mean difference 34 ms; 95% confidence interval 11–57) (Fig. 1c). Tricuspid EDT was similarly prolonged by hypoxaemia (Table 3), and tricuspid EDTc was significantly prolonged from baseline only at SaO₂ 75–80% (mean difference 33 ms; 95% confidence interval 11–55) (Fig. 1c).

Comparison of changes between left and right ventricles showed that, as a percentage of baseline

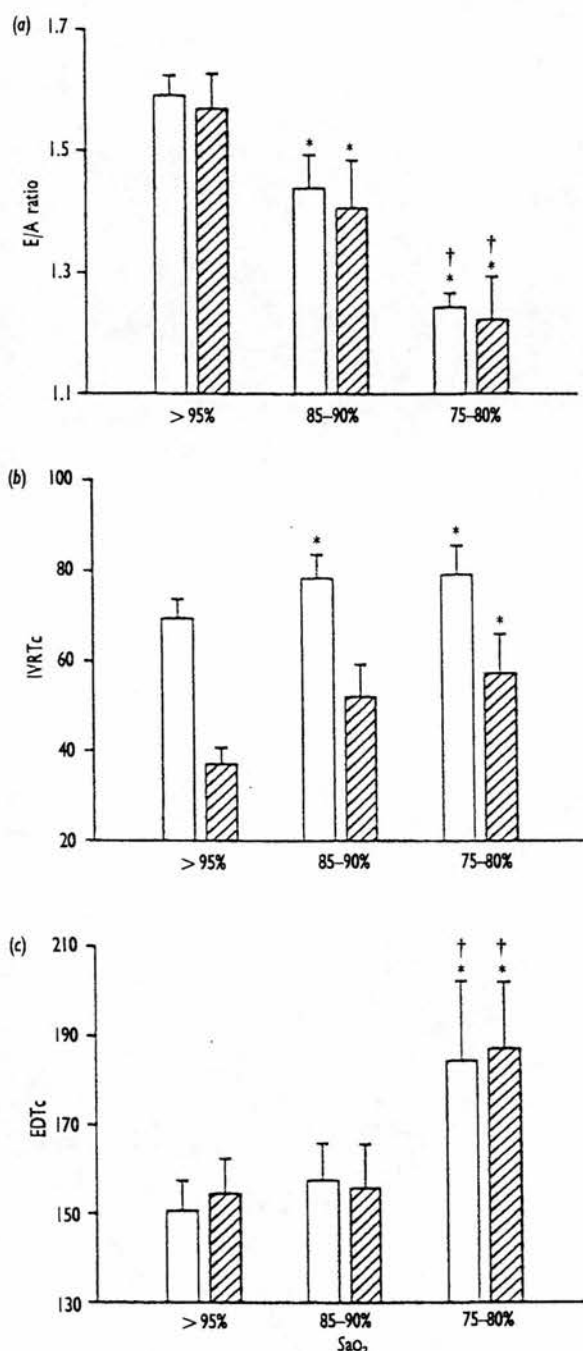


Fig. 1. Parameters of left (□) and right (▨) ventricular diastolic function showing (a) E/A velocity ratio, (b) heart rate-corrected isovolumic relaxation time (IVRTc) and (c) heart rate-corrected E-wave deceleration time (EDTc). Values are means and SEM. *Significant difference from baseline (SaO₂ > 95%). †Significant difference from SaO₂ 85–90%.

IVRTc, at SaO₂ 85–90% right ventricular IVRTc increased by 51% compared with an increase in left ventricular IVRTc of 13% (mean difference 38%; 95% confidence interval –11 to 100; not significant). At the higher level of hypoxaemia where SaO₂ was reduced to 75–80%, right ventricular IVRTc increased by 72% compared with an increase in left

Table 2. Left ventricular diastolic filling parameters. Absolute values (means \pm SEM) of maximal transmitral early ($E_{v_{max}}$) and atrial ($A_{v_{max}}$) wave velocity, E/A ratio, left ventricular isovolumic relaxation time (IVRT) and E-wave deceleration time (EDT) at each level of oxygenation. *Significantly different from >95%. †Significantly different from 85–90%.

	SaO ₂		
	>95%	85–90%	75–80%
$E_{v_{max}}$ (m/s)	0.70 \pm 0.02	0.69 \pm 0.02	0.71 \pm 0.04
$A_{v_{max}}$ (m/s)	0.44 \pm 0.01	0.48 \pm 0.02*	0.57 \pm 0.03*
E/A	1.53 \pm 0.03	1.44 \pm 0.06*	1.24 \pm 0.02*†
IVRT (ms)	70.7 \pm 6.0	74.8 \pm 6.5	72.5 \pm 7.1
EDT (ms)	146 \pm 5.6	150 \pm 8.3	168 \pm 14.4*

Table 3. Right ventricular diastolic filling parameters. Absolute values (means \pm SEM) of maximal transtricuspid early ($E_{v_{max}}$) and atrial ($A_{v_{max}}$) wave velocity, E/A ratio, right ventricular isovolumic relaxation time (IVRT) and E-wave deceleration time (EDT) at each level of oxygenation. *Significantly different from >95%. †Significantly different from 85–90%.

	SaO ₂		
	>95%	85–90%	75–80%
$E_{v_{max}}$ (m/s)	0.57 \pm 0.02	0.61 \pm 0.03	0.61 \pm 0.05
$A_{v_{max}}$ (m/s)	0.36 \pm 0.01	0.44 \pm 0.03*	0.50 \pm 0.04*
E/A	1.57 \pm 0.06	1.41 \pm 0.08*	1.22 \pm 0.07*†
IVRT (ms)	40.1 \pm 4.3	50.0 \pm 7.6	52.9 \pm 8.9*
EDT (ms)	150 \pm 6.1	151 \pm 7.0	170 \pm 8.3*

ventricular IVRTc of 14% (mean difference 58%; 95% confidence interval 6–109; $P < 0.05$).

DISCUSSION

The present study has demonstrated abnormalities of left and right ventricular filling during hypoxaemia in normal humans. While the pattern of changes in E/A ratios might conceivably be explained solely by changes in HR, the prolongation of IVRT and EDT on both sides of the heart indicate abnormal diastolic function, particularly evident when these parameters are corrected for heart rate. These haemodynamic changes are in agreement with previous work showing that chronotropic rather than inotropic effects of hypoxaemia are responsible for the increment in CO [15].

IVRT is widely accepted as a measure of active myocardial relaxation, and its prolongation indicates impaired diastolic function [16]. Conversely, EDT represents a measure of myocardial compliance and is shortened when compliance is decreased [16]. However, it is important to realize that impairment of relaxation has confounding effects on EDT. In this situation, active ventricular relaxation continues while passive filling takes place. This paradoxically increases apparent ventricular compliance with consequent lengthening of the EDT [17]. Thus, the pattern of abnormality described in the present study is one of impaired myocardial relaxation.

These findings in humans therefore confirm previously described abnormalities of myocardial relaxation observed during hypoxia in laboratory animal systems. Studies using isolated ventricular papillary muscle have demonstrated marked impairment of relaxation during hypoxia [18, 19], while in isolated intact heart preparations hypoxia is associated with an increase in ventricular chamber stiffness [20] as well as impairment of relaxation during diastole [9, 20]. The mechanisms whereby hypoxaemia might produce these effects have been extensively studied, and two possible theories are briefly considered.

Firstly, the effects of hypoxia on intracellular calcium transport may be important. Cellular hypoxia has been shown to increase intracellular calcium concentrations during diastole [9] as a result of decreased calcium uptake by the sarcoplasmic reticulum [21] and perhaps other ion channel-mediated effects [22]. Myocardial relaxation depends critically on rapid removal of free intracellular calcium ions to reverse quickly the calcium-facilitated actin-myosin interaction which would otherwise sustain contraction [23]. It is likely that hypoxia delays this process, so allowing contractile elements within the cell to remain cross-linked into diastole and impairing relaxation.

The second possibility is a mechanical one, related to the concept of ventricular interaction across the interventricular septum [6]. Hypoxaemia, by inducing pulmonary hypertension [24], increases right ventricular afterload, which might cause dynamic changes in septal motion. This pattern has been observed in other forms of pulmonary hypertension [25, 26] but has only been studied at much higher pulmonary artery pressures than we achieved. Although we did not study this phenomenon directly in the present study, it is conceivable that abnormal septal motion, which is associated with impairment of left ventricular filling [6], might explain some of the abnormalities observed.

Interestingly, the effects of pulmonary hypertension alone on right ventricular diastolic function have not been studied, although this is impaired in cor pulmonale [10], in which either pulmonary hypertension or hypoxaemia might play a role. In the present study, we have shown that, while both right and left ventricular relaxation is impaired by hypoxaemia, prolongation of IVRTc as a percentage of baseline is more marked in the right ventricle than in the left. Obviously, baseline IVRTc was shorter in the right ventricle (with values in the present study similar to those previously published although recorded by a different method [10]), but increases in pulmonary artery pressure would be expected to prolong systolic pulmonary artery flow. This would delay pulmonary valve closure and in fact shorten right ventricular IVRT. This effect would, however, need to be balanced by the possibility that increased pulmonary artery pressure would alter myocyte loading conditions in the right

ventricle, which may directly impair relaxation, as has been shown to occur in the left ventricle [27]. The finding that right ventricular IVRTc was prolonged proportionately more than left ventricular IVRTc may indicate that right ventricular relaxation is more sensitive to the adverse affects of hypoxaemia.

It would appear therefore that, as is the case *in vitro*, hypoxaemia significantly impairs myocardial relaxation in humans and that these abnormalities are of similar magnitude to the diastolic filling abnormalities observed in some disease states. IVRTc in the right ventricle was prolonged by 20.3 ms, which approaches the level of abnormality detected in patients with hypoxaemic chronic obstructive pulmonary disease and pulmonary hypertension [10]. In the left ventricle, the observed lengthening of IVRTc (by 9.8 ms) during hypoxaemia is more difficult to place in a clinical context, although is similar in magnitude to the adverse effects of very high-dose infusions of angiotensin II [28]. The haemodynamic significance of these effects is therefore speculative at present as clinical studies are lacking. Whether treating hypoxaemic patients with oxygen would actually improve diastolic function has not been studied, but it is conceivable that this is one way in which oxygen therapy is of benefit to such patients.

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Abnormal myocardial repolarisation in response to hypoxaemia and fenoterol

David G Kiely, Robert I Cargill, Alison Grove, Allan D Struthers, Brian J Lipworth

Abstract

Background – Prolongation of the QTc interval has been associated with cardiac dysrhythmias and sudden death. QTc dispersion (interlead variability in QTc interval) has recently been proposed as being a more sensitive marker of repolarisation abnormalities and shown to be a more specific index of arrhythmia risk. Although hypoxaemia and fenoterol have previously been shown to prolong the QTc interval, this does not reflect regional myocardial repolarisation abnormalities.

Methods – Electrophysiological effects were measured at baseline and after 30 minutes steady state hypoxaemia at an arterial oxygen saturation (SaO₂) of 75–80% (study 1) and at baseline then 30 minutes after inhaled fenoterol 2.4 mg (study 2). From the ECG, lead II corrected QT interval (QTc) and overall corrected QT dispersion were measured using a computer linked digitising tablet according to standard criteria.

Results – QTc dispersion was increased during hypoxia compared with baseline values (mean (SE) 69 (6) ms v 50 (5) ms) and after fenoterol compared with baseline (79 (13) v 46 (4) ms), respectively. There was also an increase in QTc interval and heart rate after fenoterol (493 (23) v 420 (6) ms and 98 (3) v 71 (6) bpm, respectively). The heart rate was increased during hypoxaemia compared with baseline (78 (3) v 64 (2) bpm), but no change occurred in the QTc interval.

Conclusions – Both hypoxaemia and fenoterol cause myocardial repolarisation abnormalities in man in terms of increased QTc dispersion, but only fenoterol increased the QTc interval. This may be relevant in the aetiology of arrhythmias in patients with acute severe asthma where β agonist therapy and hypoxaemia coexist.

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Keywords: hypoxaemia, β agonist, QT dispersion.

the recovery of ventricular excitability, widely recognised to be the most important factor in arrhythmogenesis.

Prolongation of the QT interval has been shown to be a predictor of sudden death in alcoholic cirrhosis,¹ sudden cardiovascular mortality in apparently healthy individuals,² as well as sudden death in patients with ischaemic heart disease.³ Recently, however, QTc dispersion (interlead variability in QTc interval) has been suggested as a more sensitive and specific marker as it measures differences in regional repolarisation and has been shown to be increased in patients with hypertrophic obstructive cardiomyopathy (HOCM) at risk from ventricular arrhythmias,⁴ and in patients with long QT intervals it distinguished between those with ventricular arrhythmias and those without.⁵ It has also been found to be a more sensitive and specific marker of sudden death in heart failure than QTc interval alone.⁶

Cardiac arrhythmias are a very common finding in patients with respiratory failure and chronic obstructive pulmonary disease (COPD).^{7–9} It has been shown that depressed left ventricular diastolic performance is a predictive factor for ventricular arrhythmias during respiratory failure from COPD, although the poor definition of the statistical model suggests that other factors contribute to the genesis of these arrhythmias.¹⁰ In this respect Stewart *et al*¹¹ have recently shown that QTc prolongation is a better predictor of death in patients with COPD than hypoxaemia, hypercapnia, or spirometric measurements. Interest has also surrounded the use of β agonists which have inotropic, chronotropic, and electrophysiological effects. In particular, the epidemiological observation that fenoterol was associated with an increased risk of death in patients with severe asthma^{12,13} has suggested that this drug may predispose these individuals to arrhythmias. Interestingly, the use of high dose nebulised β agonists has been associated with an increased risk of cardiac arrhythmias in patients with COPD,¹⁴ and a further study revealed that normoxaemic patients with COPD had a higher frequency of ventricular ectopy during treatment with high dose than with low dose terbutaline, although 24 hour Holter recordings did not reveal an increase in significant arrhythmias.¹⁵

We have therefore, for the first time, looked at the effects of two separate stimuli which have been suggested as important factors in arrhythmogenesis in respiratory disease –

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The surface electrocardiogram has been investigated for its ability to identify those patients at risk of arrhythmias and as such represents a cheap, non-invasive and simple method. Traditionally the single measurement of QTc interval has been used to measure

namely, hypoxia and β agonists – and their relative effects on QTc dispersion and QTc interval.

Methods

SUBJECTS

Sixteen healthy male volunteers aged 21–37 years were studied. There was no abnormality present on clinical history, clinical examination, 12 lead ECG, biochemical, or haematological screening. Patients abstained from alcohol and caffeine for a 24 hour period prior to the study. Informed written consent to the study protocol, previously approved by the Tayside committee for medical research ethics, was obtained.

STUDY PROTOCOL

Study 1

Eight subjects were studied. An intravenous cannula was inserted into the left forearm for blood sampling after which they rested supine for at least 30 minutes to obtain stable resting haemodynamics (T_0). They were then rendered hypoxaemic for 30 minutes by breathing a variable mixture of oxygen and nitrogen which rendered their arterial oxygen saturation between 75% and 80% (T_1). The hypoxic gas mixture was produced from separate cylinders of nitrogen and oxygen fitted with variable flow valves. Gases were mixed in a 25 litre Douglas bag, from which the subjects breathed through a mouthpiece connected by a series of one way

valves while wearing an occlusive nose clip. A 12 lead ECG and venous blood samples for catecholamine and serum potassium assays were taken at T_0 and T_1 .

Study 2

Eight subjects were studied. An intravenous cannula was inserted into the left forearm for blood sampling after which they rested supine for at least 30 minutes to obtain stable resting haemodynamics (T_0). Patients were then given fenoterol 2.4 mg from a metered dose inhaler (MDI) via a spacer device (12×0.2 mg actuations with three inhalations per actuation). A 12 lead ECG and venous blood samples for serum potassium were taken at T_0 and 30 minutes after inhalation of fenoterol (T_1).

MEASUREMENTS

QT interval

The ECGs from both study days were analysed in random order after completion of the study by an investigator who was not involved during either of the study days and who was blinded with respect to the stimulus the volunteers had received. If feasible, the QT interval was measured in all leads of a surface 12 lead ECG (paper speed = 25 mm/s). Three consecutive cycles were measured in each lead where possible and the mean value was taken as representing the QT interval in that lead. The QT interval was calculated according to standard

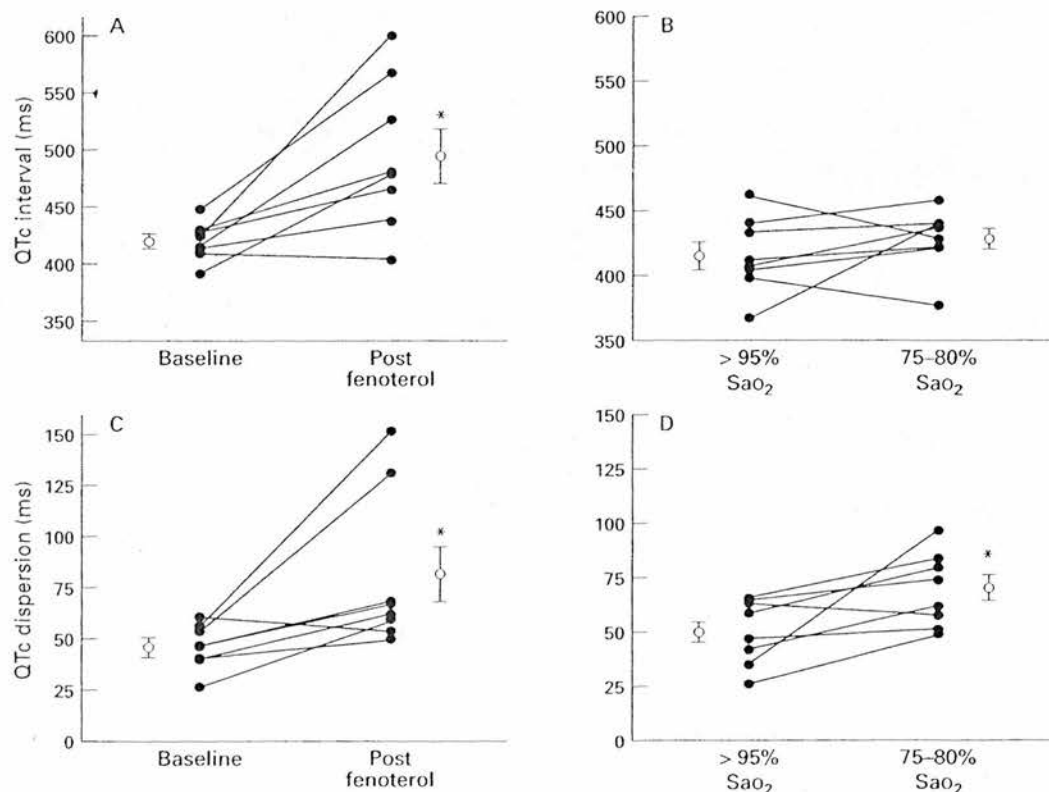


Figure 1 Effects of hypoxaemia and fenoterol on QTc interval and QTc dispersion. (A) Absolute QTc interval measured at baseline and after fenoterol; * $p < 0.01$ fenoterol treatment versus baseline. (B) Absolute QTc interval measured at baseline and during hypoxaemia. (C) Absolute QTc dispersion measured at baseline and after fenoterol; * $p < 0.05$ fenoterol treatment versus baseline. (D) Absolute QTc dispersion measured at baseline and during hypoxaemia; * $p < 0.05$ fenoterol treatment versus baseline. Values for the same patient are connected and the mean and standard error of the mean are represented as clear circles and error bars, respectively.

criteria¹⁶ from the onset of the QRS complex to the end of the T wave – that is, a return to the T/P baseline. In the presence of U waves the QT interval was measured to the nadir of the curve between the T and the U waves. QT intervals were then corrected for rate using Bazett's formula ($QTc = QT/\sqrt{RR}$).¹⁷

QTc dispersion was defined as the difference between the maximum and minimum QTc interval measured during analysis of all leads of the surface ECG.¹⁶ The measurements were made using a computer linked digitising tablet.¹⁸ To compare the standard measure of QTc interval with QTc dispersion we calculated the average of three QT intervals in lead II and corrected for heart rate using Bazett's formula ($QTc = QT/\sqrt{RR}$).

Heart rate

This was calculated from five consecutive R to R intervals in lead II.

Oxygenation

Arterial blood oxygen was continuously monitored by transcutaneous oximetry (CSI 503, Criticare Systems Inc, Waukesha, Wisconsin, USA).

Serum potassium

Samples for serum potassium were collected in chilled lithium-heparin tubes and centrifuged at 4°C immediately. The separated plasma was stored at -20°C until measured in one batch at the end of the study using an internal caesium standard flame photometer (Instrumentation Laboratory, Milan, Italy).

Catecholamines

Samples for adrenaline and noradrenaline were collected in chilled lithium-heparin tubes and centrifuged immediately at 4°C. Separated plasma was stored at -70°C and assayed in one batch at the end of the study using the double isotope radioenzymatic method.¹⁹

DATA ANALYSIS

Comparison of values between points was made by multifactorial analysis of variance (MANOVA).²⁰ A probability value of $p < 0.05$ (two tailed) was considered to be statistically significant. Data are presented as means and the standard error of the mean (SE).

Results

QTc interval

Treatment with fenoterol significantly ($p < 0.01$) increased QTc interval compared with baseline (493 (23) v 420 (6) ms; fig 1A). Hypoxaemia, however, had no significant effect (fig. 1B).

QTc dispersion

Fenoterol significantly ($p < 0.05$) increased QTc dispersion compared with baseline (79 (13) v

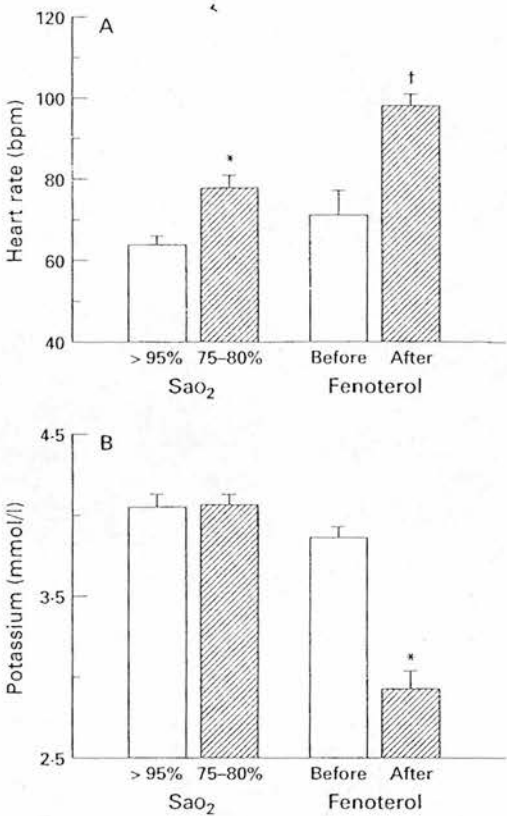


Figure 2 Effects of hypoxaemia and fenoterol on heart rate and serum potassium. (A) Left: absolute heart rate measured at baseline (□) and during hypoxaemia (▨). Right: absolute heart rate measured at baseline (□) and after fenoterol (▨). * $p < 0.05$ difference between hypoxaemia and baseline; † $p < 0.01$ difference between fenoterol and baseline. (B) Left: serum potassium measured at baseline (□) and during hypoxaemia (▨). Right: serum potassium measured at baseline (□) and after fenoterol (▨). * $p < 0.0001$ difference between fenoterol and baseline.

46 (4) ms; fig 1C). Similarly, hypoxaemia significantly ($p < 0.05$) increased QTc dispersion compared with baseline (69 (6) v 50 (5) ms; fig 1D).

Heart rate

The heart rate was significantly increased by both fenoterol (98 (3) v 71 (6) bpm; $p < 0.01$) and hypoxaemia (78 (3) v 64 (2) bpm; $p < 0.05$) compared with baseline values (fig 2A).

Serum potassium

Treatment with fenoterol resulted in a significant ($p < 0.0001$) reduction in serum potassium compared with baseline values (2.93 (0.12) v 3.86 (0.07) mmol/l). Hypoxaemia, however, had no significant effect (fig 2B).

Catecholamines

Hypoxaemia had no significant effect on either noradrenaline (4.75 (0.65) v 3.97 (0.36) nmol/l) or adrenaline (0.22 (0.02) v 0.16 (0.02) nmol/l) compared with baseline values.

Discussion

Our results show that both hypoxaemia and fenoterol significantly increase QTc dispersion in healthy volunteers, suggesting that these two stimuli cause abnormalities in myocardial repolarisation. However, only fenoterol significantly increased QTc interval and significantly decreased the serum potassium concentration compared with baseline – changes which have previously been documented in normal and asthmatic subjects.^{21 22}

It is thought that QTc dispersion reflects regional variation in ventricular repolarisation and existing evidence suggests that it is a powerful index of the propensity for developing life threatening arrhythmias.⁴⁻⁶ The underlying mechanism responsible for an increase in QTc dispersion is not known, although it has been suggested that fibrosis may be important.⁶ However, as our results suggest, dynamic physiological abnormalities must play a part as both stimuli are acute and self limiting in nature. Indeed, our results are not the first to implicate dynamic variables as having an important role in altering QTc dispersion; Moreno *et al*²³ have shown that QTc dispersion is decreased following successful thrombolysis after myocardial infarction, demonstrating that localised ischaemia affects regional repolarisation. In the context of congestive cardiac failure no correlation was noted between increased QTc dispersion and catecholamine levels.⁶ We were unable to show any increase in circulating catecholamines during acute hypoxia, although this was a small study and as such has an inherently large type 2 error. It is known that large oral doses of β agonists do not affect catecholamine levels;²⁴ they were not measured in this study although Scheinin *et al* have shown that noradrenaline was dose dependently increased by fenoterol treatment.²⁵ It may be that hypoxia and β agonists have their effects on QTc dispersion by altering autonomic tone, although it is likely that local metabolic and electrical disturbances may play a part. In this respect, hypokalaemia affects the electrical stability of cell membranes and is known to predispose individuals to arrhythmias and may explain, at least in part, why fenoterol increases QTc dispersion.

Although hypoxia did not significantly increase QTc interval in this study, this phenomenon has previously been documented.²¹ However, it may be that QTc dispersion is a more sensitive marker of abnormal myocardial repolarisation than QTc interval alone.

With respect to our methodology there are undoubtedly limitations in all studies measuring QTc dispersion. We used a computer linked digitising tablet which has been shown by other investigators to be a reliable and accurate measure of QTc dispersion.¹⁸ Probably the most important aspect concerning methodology is the protocol to define the end of the T wave. We have thus used the most commonly used protocol¹⁶ and one which has been shown to correlate with risk of arrhythmia and sudden death. We also measured the QTc interval in the majority of leads in each individual, although occasionally leads were omitted due to difficulty defining the end of the T wave. We used routine

ECGs to measure QTc dispersion since we felt this would have most clinical relevance and, indeed, no substantial evidence suggests that simultaneous ECG recording has any benefits.

What is the clinical relevance of QTc dispersion and what, if any, are its merits compared with our traditional measure of ventricular repolarisation, QTc interval? Firstly, much time and effort has been expended to find markers of mortality in hypoxic chronic lung disease and to find high risk patients who may benefit from further investigation or treatment. It would certainly be of interest if QTc dispersion correlates with risk of sudden death in patients with cor pulmonale. The use of fenoterol has been associated with an increased incidence of asthma deaths. Could it be possible that this observation is due to arrhythmias occurring in the presence of abnormal myocardial repolarisation? It has been suggested that certain individuals may be more susceptible to the effects of β agonists. In this respect it is interesting to note the large increase in QTc dispersion that occurred in two individuals in response to inhaled fenoterol (fig 1C). Although we exceeded the standard dose of fenoterol suggested by the manufacturer, in an asthmatic attack very high doses of β agonists may have to be taken²⁶ and, indeed, the British Thoracic Society guidelines recommend up to 50 puffs of a β agonist from a metered dose inhaler during a severe attack.²⁷ We have also shown that acute hypoxaemia increases QTc dispersion suggesting that this may have a part to play in arrhythmogenesis in acute asthma, although we can infer little concerning chronic hypoxia. Compared with the QTc interval, QTc dispersion, which measures ventricular repolarisation in several leads, may be a better indicator of arrhythmia risk. Indeed, it has been shown to be both a more sensitive and specific marker of sudden death than QTc interval alone.⁶ Amiodarone which is known to prolong QTc interval is used in the treatment of ventricular tachycardia. It is perhaps no coincidence that this drug also reduces QTc dispersion.²⁸

Thus, we have shown for the first time that two stimuli thought to be associated with arrhythmogenesis in respiratory disease increase QTc dispersion – namely, hypoxia and β agonists. It seems likely that this index of abnormal myocardial repolarisation will have important implications in terms of risk stratification of patients and in the development of new drugs and the investigation of current drugs in respiratory medicine.

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Effects of Hypercapnia on Hemodynamic, Inotropic, Lusitropic, and Electrophysiologic Indices in Humans*

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Study objective: The inotropic, lusitropic, and electrophysiologic effects of acute hypercapnia in humans are not known. Although the effects of hypercapnia on the systemic circulation have been well documented, there is still some debate as to whether hypercapnia causes true pulmonary vasoconstriction *in vivo*. We have therefore evaluated the effects of acute hypercapnia on these cardiac indices and the interaction of hypercapnia with the systemic and pulmonary vascular beds in humans.

Participants and interventions: Eight healthy male volunteers were studied using Doppler echocardiography. After resting for at least 30 min to achieve baseline hemodynamic parameters (T_0), they were rendered hypercapnic to achieve an end-tidal carbon dioxide (CO_2) of 7 kPa for 30 min by breathing a variable mixture of CO_2 /air (T_1). They were restudied after 30 min recovery breathing air (T_2). Hemodynamic, diastolic, and systolic flow parameters, QT dispersion (maximum-minimum QT interval measured in a 12-lead ECG), and venous blood samples for plasma renin activity (PRA), angiotensin II (ANG II), and aldosterone (ALDO) were measured at each time point.

Results: Hypercapnia compared with placebo significantly increased mean pulmonary artery pressure 14 ± 1 vs 9 ± 1 mm Hg and pulmonary vascular resistance 171 ± 17 vs 129 ± 17 dyne-s-cm⁻⁵, respectively. Heart rate, stroke volume, cardiac output, and mean arterial BP were increased by hypercapnia. Indexes of systolic function, namely peak aortic velocity and aortic mean and peak acceleration, were unaffected by hypercapnia. Similarly, hypercapnia had no effect on lusitropic indexes reflected by its lack of effect on isovolumic relaxation time, mitral E-wave deceleration time, and mitral E/A wave ratio. Hypercapnia was found to significantly increase both QTc interval and QT dispersion: 428 ± 8 vs 411 ± 3 ms and 48 ± 2 vs 33 ± 4 ms, respectively. There was no significant effect of hypercapnia on PRA, ANG II, or ALDO.

Conclusion: Thus, acute hypercapnia appears to have no adverse inotropic or lusitropic effects on cardiac function, although repolarization abnormalities, reflected by an increase in QT dispersion, and its effects on pulmonary vasoconstriction may have important sequelae in man.

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Key words: electrophysiologic; hypercapnia; inotropic; lusitropic; pulmonary circulation

Abbreviations: Acc_{mean}=aortic mean acceleration; Acc_{peak}=aortic peak acceleration; ALDO=aldosterone; ANG II=angiotensin II; Av_{max}=maximal velocity of atrial transmitral flow; Av_{peak}=aortic peak velocity; CO=cardiac output; CO_2 =carbon dioxide; DBP=diastolic arterial BP; EDT=early transmitral flow deceleration time; EDTc=early transmitral flow deceleration time adjusted for heart rate; ET CO_2 =end-tidal carbon dioxide; Ev_{max}=maximal velocity of early transmitral flow; HR=heart rate; IVRT=isovolumic relaxation time; IVRTc=isovolumic relaxation time adjusted for heart rate; MAP=mean arterial BP; MPAP=mean pulmonary artery pressure; PAT=pulmonary acceleration time; PRA=plasma renin activity; PVR=pulmonary vascular resistance; RAS=renin angiotensin system; RIA=radioimmunoassay; SBP=systolic arterial BP; SV=stroke volume; SVI=aortic systolic velocity integral; SVR=systemic vascular resistance

Hypercapnia is a well-recognized consequence of a variety of disease states. It is frequently encountered in the context of chronic obstructive airways disease and more unusually in disorders of the nervous and musculoskeletal systems. In recent years, there has been much interest in the effects of hypercapnia in anesthetic practice after the finding that mechanical

ventilation may contribute to increased morbidity and mortality as a consequence of barotrauma.¹⁻³ This has resulted in a volume- and pressure-limited ventilation strategy and elevated levels of carbon dioxide (CO_2), so-called permissive hypercapnia.^{4,5}

The effects of hypercapnia on the systemic circulation have been well documented,^{6,7} although there is still some debate as to whether CO_2 causes true pulmonary vasoconstriction *in vivo*.⁸⁻¹³ Many of these studies were performed more than 20 years ago and findings were sometimes based purely on changes in mean pulmonary artery pressure (MPAP) and where

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pulmonary vascular resistance (PVR) was measured, it was derived from cardiac outputs (COs) calculated using the Fick principle, with errors a consequence of a changing state of respiratory gas exchange.^{14,15}

The advent of newer noninvasive methods such as Doppler echocardiography has permitted a more detailed examination not only of hemodynamic effects but also of inotropic^{16,17} and lusitropic^{18,19} activity. A novel marker of abnormal myocardial repolarization, QT dispersion,^{20,21} has also provided us with information regarding the electrophysiologic effects of different stimuli.

We have therefore evaluated for the first time (to our knowledge) the effects of acute hypercapnia on inotropic, lusitropic, and repolarization indexes and reexamined the interaction between hypercapnia and the pulmonary circulation in the integrated physiologic system of man.

MATERIALS AND METHODS

Subjects

Eight healthy male volunteers, mean age 24 years (range, 21 to 34 years), were studied. There was no abnormality present on clinical history, examination, 12-lead ECG, echocardiography, biochemical screening, or hematologic screening. Informed written consent to the study protocol, previously approved by the Tayside Committee for Medical Research Ethics, was obtained.

Study Protocol

Subjects attended the clinical laboratory and were studied in a supine position, rolled slightly on the left side. An IV cannula was inserted into the left forearm for blood sampling. Subjects then rested supine for at least 30 min to obtain stable resting hemodynamics (T_0). They were then rendered hypercapnic by breathing a variable mixture of CO₂ and medical air to attain an end-tidal CO₂ (ETCO₂) of 7 kPa for 30 min (T_1) and then they breathed room air for a further 30 min (T_2). The hypercapnic gas mixture was produced from separate cylinders of CO₂ and medical air fitted with variable flow valves. Cases were mixed in a 25-L Douglas bag (Collins Inc; Braintree, Mass) from which the subjects breathed through a mouthpiece connected by a series of one-way valves, while wearing an occlusive nose clip. Measurements of pulmonary and systemic hemodynamic variables, inotropic, lusitropic, and electrophysiologic indexes, and venous blood samples for plasma renin activity (PRA), angiotensin II (ANG II), and aldosterone (ALDO) were taken at T_0 , T_1 , and T_2 .

Measurements

Oxygenation: Arterial blood oxygen saturation was continuously monitored by transcutaneous oximetry (CSI 503; Criticare Systems Inc; Waukesha, Wis). Recordings were averaged at steady state over a period of 5 min at each time point for the purpose of analysis.

End-tidal CO₂: This was measured continuously with the tip of the gas sampling tube adjacent to the mouth of the subject, using a transportable ETCO₂ monitor (POET TE; Criticare Systems Inc; Waukesha, Wis). Recordings at steady state were averaged over a period of 5 min and this value was used for the purpose of analysis.

Hemodynamics: Systolic arterial BP (SBP), mean arterial BP (MAP) and diastolic arterial BP (DBP) were measured using a semiautomatic sphygmomanometer (Vital Signs Monitor; Critikon;

Tampa, Fla). The mean of three consistent readings was taken at each time point. Heart rate (HR) was recorded on an ECG trace and an average rate over 6 R-R intervals was calculated. Pulmonary acceleration time (PAT) in milliseconds was measured as previously described^{22,23} from pulmonary arterial flow by pulsed-wave Doppler echocardiography (Vingmed SD50; Vingmed Sound; Horten, Norway) from the left third/fourth intercostal space. The mean of three consistent waveforms at each time point was used for the purpose of analysis. MPAP in mm Hg was calculated as $MPAP = 73 - (0.42 \times PAT)$.²⁴ Aortic cross-sectional area was measured by M-mode echocardiography (Vingmed SD50). The aortic systolic velocity integral (SVI) was measured by on-line computer-assisted determination using pulsed-wave Doppler echocardiography of ascending aortic blood flow from the suprasternal notch. On-line calculations of stroke volume ($SV = SVI \times \text{cross-sectional area}$) and CO as the product of SV and HR were also made. Total PVR was calculated as: $PVR = MPAP / CO \times 80 \text{ dyne} \cdot \text{s} \cdot \text{cm}^{-5}$. We have previously shown the short term coefficients for measurement of PAT and SVI to be 1.7% and 1.2%, respectively.²²

Systolic Flow Parameters: Doppler ascending aortic blood flow (Vingmed SD50) was recorded with a 2.0-MHz pulsed-wave transducer with depth adjusted to give maximal velocity and the following variables were measured: aortic peak acceleration (Acc_{peak}), aortic mean acceleration (Acc_{mean}), and aortic peak velocity (Av_{peak}). We have previously shown the coefficient of variability for the measurement of Acc_{mean} and Av_{peak} by this method to be 12.5% and 4.4%, respectively.²²

Diastolic Filling Parameters: From the apical window, pulsed-wave Doppler analysis of mitral and diastolic flow was combined with simultaneous phonocardiogram recording with the microphone (Siemens AG; Munich, Germany). Measurements were all made on-line during expiration and in triplicate, with a display sweep speed of 100 mm/s. Transmitral flow was analyzed after adjusting sample volume depth to yield maximal E-wave velocities with clearly defined flow velocity envelopes. Measurement of diastolic flow parameters from these signals has previously been shown to be highly reproducible and easily applicable in our own laboratory¹⁹ and also by other workers.²⁵ The aortic component of the second heart sound was identified on the phonocardiogram trace by noting closure artifacts from superimposition of aortic Doppler flow profiles. From diastolic transmitral flow, maximal velocities of the early (Ev_{max}) and atrial (Av_{max}) components of flow were measured, and the ratio of Ev_{max} and Av_{max} (E/A ratio) was calculated. In addition, the E-wave deceleration time (EDT) was calculated as the time in milliseconds from peak velocity to the end of the E wave. The isovolumic relaxation time (IVRT) was calculated for the left ventricle as the time in milliseconds from the aortic component of the phonocardiogram second heart sound to the onset of diastolic transmitral flow. Both EDT and IVRT were corrected for changes in HR induced by hypoxemia by dividing by the square root of the simultaneous ECG R-R interval; $EDT_c = IVRT / \sqrt{RR}$, $IVRT_c = IVRT / \sqrt{RR}$.

QT Interval Measurement: The ECGs from both study days were analyzed in random order after completion of the study, by an investigator who was blinded with respect to the stimulus the volunteers had received. QT interval if feasible was measured in all leads of a surface 12-lead ECG (paper speed=25 mm/s). Three consecutive cycles were measured in each lead where possible and the mean value was taken as representing the QT interval in that lead. QT interval was calculated according to standard criteria²⁰ from the onset of the QRS complex to the end of the T wave *ie*, a return to the T/P baseline. In the presence of U waves, the QT interval was measured to the nadir of the curve between the T and the U waves.

QT dispersion was defined as the difference between the max-

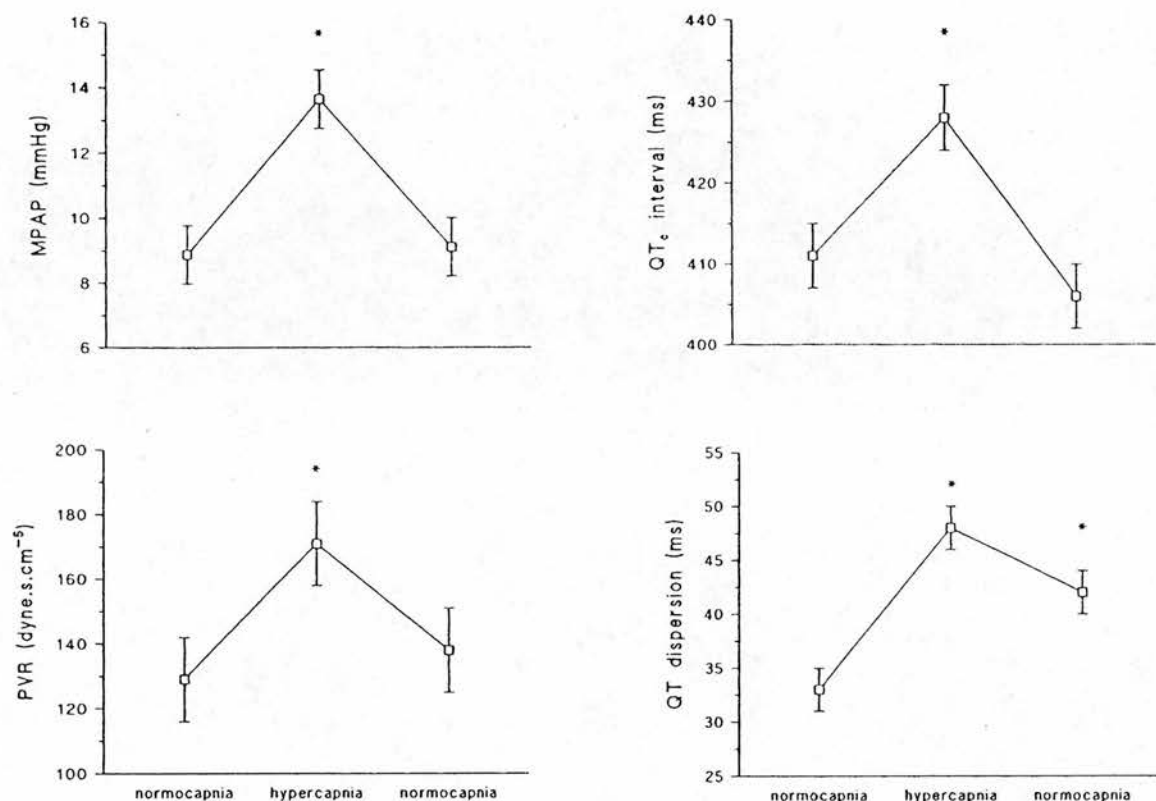


FIGURE 1. Effects of hypercapnia on pulmonary hemodynamics and electrophysiologic parameters. *Top left*: absolute MPAP measured during normoxemia (baseline), after 30 min hypercapnia, and 30 min after rebreathing air, respectively. *Bottom left*: absolute PVR measured during normoxemia (baseline), after 30 min hypercapnia, and 30 min after rebreathing air, respectively. *Top right*: absolute QTc interval measured during normoxemia (baseline), after 30 min hypercapnia, and 30 min after rebreathing air, respectively. *Bottom right*: absolute QT dispersion measured during normoxemia (baseline), after 30 min hypercapnia, and 30 min after rebreathing air, respectively. Asterisk=a significant ($p < 0.05$) difference between baseline and hypercapnia or between baseline and after 30 min rebreathing air.

imum and minimum QT interval measured during analysis of all leads of the surface ECG.²⁰ The measurements were made using a computer-linked digitizing tablet.²⁶ To compare the standard measure of QT interval with QT dispersion, we calculated the average of six QT intervals in lead II. QT intervals were then corrected for rate using the formula of Bazett²⁷ ($QTc = QT/\sqrt{RR}$).

RAS Activity: Venous blood samples for plasma ALDO were collected into lithium heparin tubes, and for measurement of plasma renin activity (PRA) into edetic acid tubes, before being centrifuged; plasma was stored at -20°C until assayed. PRA was assayed using commercially available radioimmunoassay (RIA) kits (Sorin Biomedica; Saluggia, Italy) that assayed PRA by measurement of amount of angiotensin I generated per hour. ALDO was measured using a similar RIA assay kit (Sorin Biomedica). Samples for ANG II assay were collected into chilled glass tubes containing 0.5 mL of a cocktail comprising 0.05 mmol/L 0-phenanthroline, 0.2 g neomycin, 0.125 mmol/L edetic acid, and 2% (vol/vol) alcohol before centrifugation, and separated plasma was stored at -70°C . ANG II assay was performed following separation from plasma proteins by alcohol extraction using a specific commercially available RIA kit (Nichols Institute Diagnostics; San Juan Capistrano, Calif). We have previously shown the coefficients of variation for analysis were as follows: ANG II 11.2%; PRA, 7.6%; and ALDO, 8.3%.²⁸

Data Analysis

Comparisons between serial time points on the same study day were made using multifactorial analysis of variance followed by Duncan's multiple range test. A probability value of $p < 0.05$ (two-tailed) was considered to be statistically significant. Data are presented in the text, tables, and figures as means and SEM.

RESULTS

Oxygenation and ETCO_2

Breathing the CO_2/air mixture compared to air significantly increased respiratory rate 21 ± 1 vs 13 ± 1 breaths/min, ETCO_2 7.0 ± 0.2 vs 5.0 ± 0.3 kPa, and oxygen saturation 98 ± 0.2 vs $97 \pm 0.2\%$, respectively. There was no significant difference between T_2 (30 min posthypercapnia) and baseline.

Pulmonary Hemodynamics

Hypercapnia (T_1) was associated with a significant ($p < 0.05$) increase in both MPAP and PVR compared

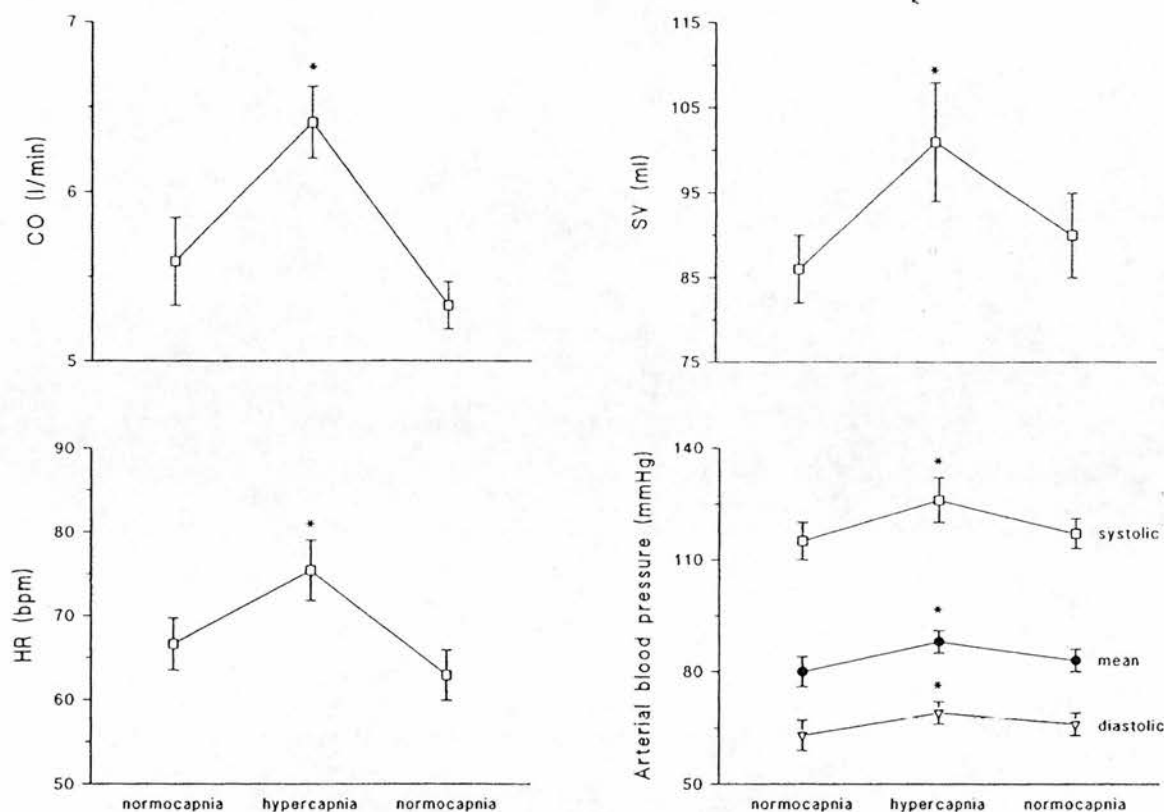


FIGURE 2. Effects of hypercapnia on systemic hemodynamic parameters. *Top left*: absolute CO measured during normoxemia (baseline), after 30 min hypercapnia, and 30 min after rebreathing air, respectively. *Bottom left*: absolute HR measured during normoxemia (baseline), after 30 min hypercapnia, and 30 min after rebreathing air, respectively. *Top right*: absolute SV measured during normoxemia (baseline), after 30 min hypercapnia, and 30 min after rebreathing air, respectively. *Bottom right*: absolute SBP (open squares), MAP (solid circles), and DBP (open triangles) measured during normoxemia (baseline), after 30 min hypercapnia, and 30 min rebreathing air, respectively. Asterisk=a significant ($p < 0.05$) difference between baseline and hypercapnia.

with baseline (T_0) (Fig 1). There was no significant difference between T_2 (30 min posthypercapnia) and baseline.

Systemic Hemodynamics

Hypercapnia (T_1) was associated with a significant ($p < 0.05$) increase in SBP, DBP, MAP, HR, and CO compared with baseline (T_0) (Fig 2). However, hypercapnia had no significant effect on systemic vascular

resistance (SVR) compared with baseline: $1,102 \pm 38$ vs $1,162 \pm 78$ dyne-s-cm⁻⁵. There was no significant difference between T_2 and T_0 for any of the systemic hemodynamic parameters.

Systolic Flow Parameters

Hypercapnia compared with baseline had no significant effect on Av_{peak} , Acc_{peak} , or Acc_{mean} (Table 1).

Table 1—Hypercapnia and Its Effects on Systolic and Diastolic Parameters*

	T_0	T_1	T_2
Av_{peak} , ms ⁻¹	1.20 ± 0.08	1.26 ± 0.10	1.15 ± 0.05
Acc_{mean} , ms ⁻²	11.9 ± 1.4	10.8 ± 1.2	10.8 ± 1.1
Acc_{peak} , ms ⁻²	26.8 ± 4.0	24.8 ± 3.6	28.1 ± 3.6
Ev_{max} , ms ⁻¹	77 ± 5	75 ± 6	71 ± 5
Av_{max} , ms ⁻¹	42 ± 2	41 ± 3	41 ± 3
E/A ratio	1.86 ± 0.17	1.90 ± 0.21	1.81 ± 0.19
EDT, ms	121 ± 5	123 ± 7	114 ± 9
EDTc, ms	121 ± 9	137 ± 9	124 ± 5
IVRT, ms	66 ± 5	65 ± 4	68 ± 3
IVRTc, ms	74 ± 5	70 ± 3	72 ± 4

*There were no significant differences between T_0 (baseline), T_1 ($ETCO_2 = 7$ kPa), and T_2 (after rebreathing air for 30 min) for each of the above variables.

Table 2—Hypercapnia and Its Effects on the RAS

	T ₀	T ₁	T ₂
PRA, pmol/l/mL	1.21±0.31	1.00±0.21	0.57±0.15*
ANG II, pmol/L	15.8±2	19.0±4.7	13.2±1.4
ALDO, pmol/L	86.2±14.7	74.49±11.6	72.3±16.3

*A significant difference in PRA at T₂ (30 min after rebreathing air) compared with T₀ (baseline). There were no significant differences between T₁ (ETCO₂=7 kPa) and the other time points for any of the above variables.

Diastolic Flow Parameters

Similarly hypercapnia compared with baseline had no significant effect on $E_{v_{max}}$, $A_{v_{max}}$, E/A ratio, EDT, EDTc, IVRT, or IVRTc (Table 1).

QT Dispersion

Hypercapnia compared with baseline had no significant effect on QT interval, although QTc was significantly increased after hypercapnia (Fig 1). Hypercapnia also significantly increased QT dispersion compared with baseline and this was also significantly elevated after 30 min rebreathing air compared with baseline.

Renin Angiotensin System (RAS) Activity

Hypercapnia had no significant effect on ANG II, PRA, or ALDO, although PRA was significantly lower at T₂ with baseline (Table 2).

DISCUSSION

We have shown that acute hypercapnia causes true pulmonary vasoconstriction *in vivo* in normal volunteers as reflected by a significant increase in both MPAP and PVR. Although acute hypercapnia had no significant inotropic or lusitropic effects, it significantly increased QT dispersion, suggesting that hypercapnia may cause abnormalities in myocardial repolarization.

The effect of CO₂ on the pulmonary circulation in man remains controversial, although the evidence appears to suggest a vasoconstrictor effect.⁸⁻¹³ We aimed to achieve ETCO₂ similar to that encountered in patients with exacerbations of COPD and also that found in permissive hypercapnia. Our mean ETCO₂ of 7 kPa equates with an arterial Pco₂ of approximately 7.5 kPa, and ETCO₂ is known to closely mirror the concentration of CO₂ in arterial blood.²⁹ Blood leaving the ventilated alveoli usually mixes with blood from both parenchymal lung tissue and with blood passing through nonventilated alveoli, creating a venous admixture. It is this venous admixture that accounts for the normal alveolar-arterial CO₂ tension difference. The early work of Fishman et al⁹ looked at the effect of 3 to 5% CO₂ on the pulmonary vasculature in normal volunteers and in patients with COPD and concluded that breathing air rich in CO₂ had no effect on pulmonary vasoconstriction. This was in sharp contrast to work performed in animals and this apparent dichotomy was explained by Kilburn et al⁸

who demonstrated pulmonary vasoconstriction in patients with COPD exposed to more severe hypercapnia. These findings have been corroborated in other studies in patients with elevated and normal MPAPs.^{10,30} This study in normal humans provides further support for the evidence in patient studies that hypercapnia is a relatively weak pulmonary vasoconstrictor and that pulmonary vessels may be the exception to the rule that acidosis causes vasodilatation.³¹ Thus, hypercapnia may function in humans as an intrinsic mechanism diverting blood from underventilated areas of the lung in an effort to maintain ventilation perfusion matching. In contrast to previous studies, we have used Doppler echocardiography to measure hemodynamic changes in the pulmonary circulation. These noninvasive techniques have been shown to be highly reproducible²² and the close correlation between Doppler PAT and MPAP as measured by right heart catheter is well established.^{24,32,33} We looked at two measures of pulmonary vasoconstriction: changes in MPAP and PVR. The use of total PVR does not account for any changes in the postcapillary vascular bed, as conventionally assessed by pulmonary capillary wedge pressure. In this respect we believe that it is unethical to insert Swan-Ganz catheters into normal volunteers for research purposes and the extra information this would give us is not essential. It has previously been shown that hypercapnia has no significant effects on pulmonary capillary wedge pressure either in patients with normal pulmonary artery pressures or those with elevated pressures occurring as a consequence of hypoxic lung disease and so effects on total PVR are reflective of changes in true PVR in precapillary arterioles during hypercapnia.¹⁰ We believe, therefore, that the observed changes in total PVR are a true reflection of changes in pulmonary vascular tone.

The systemic effects of hypercapnia are complex and reflect a balance between the direct effects of CO₂ and the secondary effects of CO₂ mediated via the central and autonomic nervous systems. In this study, we have demonstrated significant increases in HR, SV, CO, SBP, MAP, and DBP and a nonsignificant reduction in SVR, changes that have previously been documented in patients with similar degrees of hypercapnia.^{6,7}

Interestingly, although hypercapnia has been shown to be a direct myocardial depressant in the isolated

heart,^{34,35} we have shown no significant effects of hypercapnia on either inotropic or lusitropic indexes of cardiac function measured using Doppler echocardiography. Mean and peak aortic acceleration as well as peak aortic velocity have been shown to be sensitive markers of left ventricular contractility.^{16,36-38} Although Acc_{peak} and Av_{peak} decline with increasing HR³⁹ during pacing, the effects across our HR range are small and consistent with the nonsignificant changes observed. This suggests that the systolic contractility of the normal human myocardium is relatively resistant to the effects of acute hypercapnia. Similarly we have shown no effect on ventricular diastolic function, which is an important determinant of overall cardiovascular performance and a sensitive marker of cardiac dysfunction, as reflected by the lack of effect of hypercapnia on all of the measured indexes. This suggests that the secondary effects of hypercapnia on the central and autonomic nervous systems in the integrated physiologic system of humans are capable of antagonizing the direct myocardial depressant effects of hypercapnia.^{40,41}

Although acute hypercapnia appears to have no significant effects on myocardial contractility, the observation that hypercapnia increases both QTc interval and QT dispersion suggests that it has significant effects on myocardial repolarization. The finding that hypercapnia increases QT dispersion is probably of more significance since this represents differences in regional myocardial repolarization and as such represents a putative substrate for arrhythmias. In contrast, QTc interval provides no information regarding regional repolarization abnormalities. Evidence suggests that QT dispersion is a sensitive index of the propensity for developing life-threatening arrhythmias and as such may lower the arrhythmogenic threshold in conditions in which hypercapnia exists. The mechanism whereby hypercapnia causes these abnormalities in myocardial repolarization may be related to its effects on autonomic function or elevated levels of catecholamines that have been previously demonstrated during acute hypercapnia.⁴² QT dispersion is a useful, easily applicable noninvasive technique. We used a computer-linked digitizing tablet that has been shown by other investigators to be a reliable and accurate measure of QT dispersion.²⁶ Probably the most important aspect concerning methods is the protocol to define the end of the T wave. We have thus used the most commonly used protocol²⁰ and one that has been shown to correlate with arrhythmia risk and sudden death in patient studies.^{43,44} We used routine ECGs to measure QT dispersion because we believed that this would have the most clinical relevance, and indeed no substantial evidence suggests that simultaneous ECG recording has any benefits.

We have also investigated the effect of acute hypercapnia on the RAS. In the absence of hypercapnia, RAS activation in hypoxemic patients is rare,⁴⁵ suggesting a possible role for hypercapnia possibly occurring as a consequence of renal vasoconstriction or as a result of a direct cellular effect. In this study, however, we were unable to demonstrate any significant effect of hypercapnia on PRA, ANG II, or ALDO. This may be related to the brevity of our stimulus, although similar periods of hypoxia suppressed ALDO levels.⁴⁶ It is also possible that hypoxia and hypercapnia may need to be present in synergistic fashion to produce clinically detectable RAS activation. The significant fall in PRA 30 min after cessation of hypercapnia compared with baseline is consistent with the known effects of resting in the supine position, in which values of PRA increase with upright body posture and fall with time when the supine position is assumed.^{47,48}

To conclude, acute hypercapnia appears to have no effects on myocardial contractility or relaxation in the integrated physiologic system of humans, although repolarization abnormalities reflected by an increase in QT dispersion may provide an environment for arrhythmogenesis. We have also shown that hypercapnia causes true pulmonary vasoconstriction in humans. This agrees with findings in patient studies but also suggests that *in vivo*, hypercapnia has a role to play in modulating pulmonary blood flow in healthy humans.

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Angiotensin II Receptor Blockade and Effects on Pulmonary Hemodynamics and Hypoxic Pulmonary Vasoconstriction in Humans*

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Study objective: We examined the hypothesis that angiotensin II (ANG II) is a modulator of pulmonary vascular tone by examining the effects of ANG II blockade on pulmonary hemodynamics during normoxemia and hypoxemia in normal volunteers with an activated renin angiotensin system (RAS).

Participants and interventions: Eight normal volunteers, pretreated with furosemide, were studied on two separate occasions and received either an infusion of saralasin, 5 µg/kg/min, or placebo. After 20 min, they were rendered hypoxemic, by breathing N₂/O₂ mixture for 20 min to achieve arterial oxygen saturation (SaO₂) of 85 to 90% adjusted for a further 20 min to achieve SaO₂ of 75 to 80%. Doppler echocardiography was used to measure mean pulmonary artery pressure (MPAP), cardiac output, and hence total pulmonary vascular resistance (TPR).

Results: Saralasin compared with placebo resulted in a significant ($p < 0.05$) reduction in MPAP during normoxemia, 6.70 ± 1.0 vs 11.7 ± 1.3 mm Hg; at SaO₂ of 85 to 90%, 14.7 ± 1.4 vs 20.5 ± 1.0 mm Hg; and at SaO₂ of 75 to 80%, 18.1 ± 1.9 vs 27.8 ± 1.9 mm Hg, respectively. Likewise saralasin compared with placebo resulted in a significant reduction in TPR during normoxemia, 104 ± 14 vs 180 ± 20 dyne·s·cm⁻⁵; at SaO₂ of 85 to 90%, 222 ± 24 vs 295 ± 21 dyne·s·cm⁻⁵; and at SaO₂ of 75 to 80%, 238 ± 21 vs 362 ± 11 dyne·s·cm⁻⁵, respectively. The Δ MPAP response to hypoxemia was likewise significantly ($p < 0.01$) attenuated by saralasin infusion compared with placebo: mean difference 5.0 mm Hg, 95% confidence interval (CI) 1.9 to 8.08, and there was a trend toward attenuation of the Δ TPR response to hypoxemia ($0.05 < p < 0.10$): mean difference 47 dyne·s·cm⁻⁵, 95% CI, -10 to 105.

Conclusion: In addition to causing pulmonary vasodilatation in the presence of an activated RAS, our results suggest that ANG II receptor blockade attenuates acute hypoxic pulmonary vasoconstriction and that ANG II may play a role in modulating this response in normal man.

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Key words: angiotensin II blockade; hypoxemia; pulmonary circulation

Abbreviations: ACE=angiotensin-converting enzyme; ANG II=angiotensin II; CI=confidence interval; CO=cardiac output; CSA=cross-sectional area; HPV=hypoxic pulmonary vasoconstriction; HR=heart rate; MAP=mean arterial BP; MPAP=mean pulmonary artery pressure; PAT=pulmonary acceleration time; PCWP=pulmonary capillary wedge pressure; PRA=plasma renin activity; RAS=renin angiotensin system; RIA=radioimmunoassay; SaO₂=arterial oxygen saturation; SV=stroke volume; SVI=aortic systolic velocity integral; SVR=systemic vascular resistance; TPR=total pulmonary vascular resistance

In man, hypoxemia usually arises as a result of pulmonary disease or adverse environmental conditions such as altitude. The effects of hypoxemia on

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the pulmonary vasculature have been studied extensively since Von Euler and Liljestrand¹ first described the phenomenon of hypoxic pulmonary vasoconstriction (HPV).

Although HPV has beneficial effects, the stimulus of chronic hypoxia results in an elevation of pulmonary artery pressure leading to the development of cor pulmonale.^{2,3}

These patients have activation of the renin angiotensin system (RAS),^{4,6} with elevated levels of angiotensin II (ANG II). ANG II and hypoxia have both been shown to be potent pulmonary vasoconstrictors in man.⁷ *In vitro* studies have shown that ANG II facilitates HPV in the rat and potentiates this response in dogs.^{8,9} Furthermore, the use of angiotensin-converting enzyme (ACE)-inhibitors in chronically hypoxic rats has been shown to attenuate the development of pulmonary hypertension.¹⁰

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Studies in humans are rare, although recent data have shown that pretreatment with the long-acting ACE inhibitor lisinopril blunts acute HPV in normal volunteers compared to placebo.¹¹ The effect of ACE inhibition could in theory be due to lowering levels of ANG II (a vasoconstrictor) or augmenting levels of bradykinin (a vasodilator) by suppressing the activity of kininase II, which normally contributes to the degradation of kinins.¹²

The purpose of this study was to determine the role of ANG II in the pulmonary circulation during both normoxemia and hypoxemia by competitive inhibition of ANG II with its analogue, saralasin (1-sar-5-val-8-ala-ANG II), which has been shown to be a competitive antagonist of ANG II in the presence of an activated RAS, but has no effect on bradykinin degradation.¹³

MATERIALS AND METHODS

Subjects

Eight healthy male volunteers, age (mean \pm SEM) 29 ± 3 years, were studied on 2 separate occasions. There was no abnormality present on clinical history, examination, 12-lead ECG, echocardiography, biochemical screening, or hematologic screening. No medications were permitted during and for 1 month prior to the study. Informed written consent to the study protocol, previously approved by the Tayside Committee for Medical Research Ethics, was obtained.

Study Protocol

Subjects attended the laboratory at the same time of the day on 2 separate occasions, at least 1 week apart. Subjects were pretreated with 4 daily doses of furosemide, 40 mg, to activate the RAS such that saralasin would function as a pure antagonist of ANG II devoid of partial agonist activity. An indwelling IV cannula was inserted into each antecubital fossa, one for infusion of saralasin or placebo and the other for blood sampling. Subjects then rested in a supine position for 30 min to obtain stable resting hemodynamics (T_0). They then received either an infusion of 5% dextrose or saralasin 5 μ g/kg/min (Sigma Chemical Company; St Louis). They were restudied after 20 minutes (T_1), and were then rendered hypoxemic by breathing a variable mixture of oxygen and nitrogen sufficient to render arterial oxygen saturation (SaO_2) between 85 and 90% (T_2) for 20 min adjusted for a further 20 min to achieve an SaO_2 of 75 to 80% (T_3). The hypoxic gas mixture was produced from separate cylinders of nitrogen and oxygen fitted with variable flow valves. Gases were mixed in a 25-L Douglas bag, from which the subjects breathed through a mouthpiece connected by a series of one-way valves, while wearing an occlusive nose clip. Measurements of pulmonary and systemic hemodynamic variables and venous blood samples for plasma renin activity (PRA) were taken at T_0 , T_1 , T_2 , and T_3 . Serum electrolytes were measured at T_0 .

Measurements

Oxygenation: Arterial blood oxygen saturation was continuously monitored by transcutaneous oximetry (CSI 503; Criticare Systems Inc; Waukesha, Wis).

Hemodynamics: Heart rate (HR) and mean arterial BP (MAP) was measured by semiautomatic sphygmomanometer as the mean of three consecutive readings (Vital Signs Monitor; Critikon; Tampa, Fla). Pulmonary acceleration time (PAT) in milliseconds was measured as previously described¹⁴ from pulmonary arterial

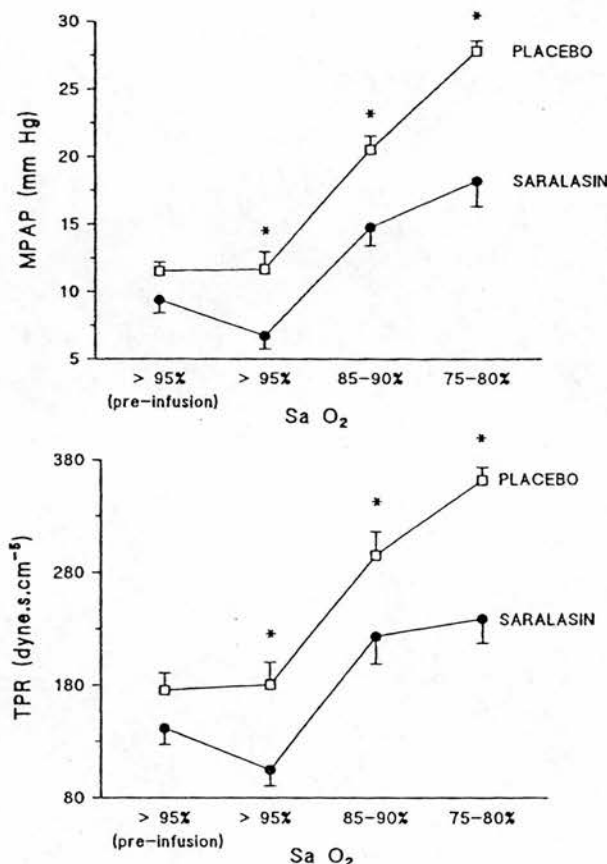


FIGURE 1. Pulmonary hemodynamic response to ANG II blockade during normoxemia and hypoxemia. *Top:* absolute Doppler MPAP measured at baseline before infusion with normoxemia, during infusion of placebo or saralasin with normoxemia, during infusion with hypoxemia (SaO_2 85 to 90%), and during infusion with hypoxemia (SaO_2 75 to 80%). Asterisk represents a significant ($p < 0.05$) difference between placebo and saralasin at each time point. *Bottom:* absolute TPR measured at baseline before infusion with normoxemia, during infusion of placebo or saralasin with normoxemia, during infusion with hypoxemia (SaO_2 85 to 90%), and during infusion with hypoxemia (SaO_2 75 to 80%). Asterisk represents a significant ($p < 0.05$) difference between placebo and saralasin at each time point.

flow by pulsed-wave Doppler echocardiography (Vingmed SD50; Vingmed Sound; Horten, Norway) from the left third/fourth intercostal space. The mean of three consistent waveforms at each time point was used for the purpose of analysis. Mean pulmonary artery pressure (MPAP) (mm Hg) was calculated as $MPAP = 73 - (0.42 \times PAT)$.¹⁵ Aortic cross-sectional area (CSA) was measured by M-mode echocardiography (Vingmed SD50). The aortic systolic velocity integral (SVI) was measured by on-line computer-assisted determination using pulsed-wave Doppler echocardiography of ascending aortic blood flow from the suprasternal notch. On-line calculations of stroke volume ($SV = SVI \times CSA$) and cardiac output (CO) as the product of SV and HR were also made. Total pulmonary vascular resistance (TPR) was calculated as follows: $TPR = MPAP / CO \times 80$ dyne.s.cm⁻⁵. We have shown previously the short-term coefficients of variability for measurement of PAT and SVI in our hands to be 1.7% and 1.2%, respectively.¹⁴

Electrolytes: Samples for serum electrolytes were collected in chilled lithium-heparin tubes and were centrifuged at 4°C immediately and separated plasma was stored at -20°C until measured in one batch at the end of the study using an internal caesium

standard flame photometer (Instrumentation Laboratory; Milan, Italy).

RAS Activity: Samples for measurement of plasma renin activity (PRA) were collected into chilled EDTA tubes. They were centrifuged at 4°C immediately and separated plasma was stored at -20°C until assayed in one batch at the end of the study. PRA was assayed using commercially available radioimmunoassay (RIA) kits (Sorin Biomedica; Saluggia, Italy) that assayed PRA by measurement of amount of angiotensin I generated per hour.

Data Analysis

Comparison of values between study days or between serial time points on the same study day was made by multifactorial analysis of variance.¹⁶ A probability value of $p < 0.05$ (two-tailed) was considered to be statistically significant. Data are presented in the text, tables, and figures as means and SEM, and where a difference between means is quoted, the 95% confidence interval (CI) for this difference is given.

RESULTS

Pulmonary Hemodynamics

There was no significant difference in PAT, MPAP, or TPR at baseline (T_0) between study days. Infusion of saralasin compared to the placebo resulted in a significant ($p < 0.05$) reduction in MPAP during normoxemia (T_1), mean difference 4.6 mm Hg (95% CI, 1.25 to 8.0); at an SO_2 of 85 to 90% (T_2), mean difference 6.1 mm Hg (95% CI, 1.4 to 10.8); and a significant ($p < 0.0005$) difference at an SO_2 of 75 to 80% (T_3), mean difference 9.6 mm Hg (95% CI, 6.0 to 13.2), respectively (Fig 1, top). Likewise saralasin infusion compared to placebo resulted in a significant ($p < 0.05$) reduction in TPR at T_1 , mean difference 76 dyne·s·cm⁻⁵ (95% CI, 29 to 123); at T_2 , mean difference 72 dyne·s·cm⁻⁵ (95% CI, 8 to 136); and a significant ($p < 0.0005$) reduction at T_3 , mean difference 123 dyne·s·cm⁻⁵ (95% CI, 82 to 165), respectively (Fig 1,

bottom). Hypoxemia caused a significant ($p < 0.05$) increase in MPAP and TPR at T_2 and T_3 compared to baseline on both study days. Saralasin compared to placebo significantly reduced PAT during normoxemia and moderate and severe hypoxemia (Table 1).

In terms of change in MPAP (Δ MPAP) from baseline (T_0) to severe hypoxemia (T_3), the Δ MPAP response was significantly ($p < 0.005$) attenuated by saralasin infusion compared with placebo: mean difference 7.3 mm Hg; 95% CI, 3.9 to 10.7. Likewise the Δ TPR response from T_0 to T_3 was significantly ($p < 0.05$) attenuated by saralasin infusion compared with placebo: mean difference 89 dyne·s·cm⁻⁵; 95% CI, 26 to 152. Because there was a significant fall in MPAP and TPR at T_1 compared with baseline (T_0) after saralasin infusion compared to placebo, we also assessed the Δ MPAP and Δ TPR response from T_1 to T_3 . The Δ MPAP response was likewise significantly ($p < 0.01$) attenuated by saralasin infusion compared with placebo: mean difference 5.0 mm Hg; 95% CI, 1.9 to 8.08; and there was a trend toward attenuation of the Δ TPR response from T_1 to T_3 ($0.05 < p < 0.10$): mean difference 47 dyne·s·cm⁻⁵; 95% CI, -10 to 105.

Systemic Hemodynamics

Although, saralasin infusion compared to placebo did not significantly alter systemic hemodynamic parameters either at baseline or during hypoxia, saralasin infusion had significant effects on systemic hemodynamics compared with baseline measurements (Table 1). Compared to baseline (T_0), a significant ($p < 0.05$) increase in CO in response to hypoxemia (T_3) was noted on both study days. A fall in systemic vascular resistance (SVR) in response to hypoxemia (T_3) was

Table 1—Effects of ANG II Receptor Blockade on Systemic Hemodynamics and PAT*

SaO ₂	Preinfusion >95% (T_0)	Infusion		
		>95% (T_1)	85-90% (T_2)	75-80% (T_3)
HR, beats/min				
P	71.0±4.8	68.6±4.2	73.1±4.3	73.8±5.6
S	67.2±3.8	64.1±3.5	68.5±4.5	71.8±5.1
MAP, mm Hg				
P	84.8±4.5	81.9±3.5	82.4±3.8	86.5±3.1
S	83.4±3.0	76.9±1.9 [†]	79.4±3.3	77.4±4.2 [†]
CO, L/min				
P	5.45±0.48	5.20±0.25	5.69±0.38	6.18±0.24 [†]
S	5.36±0.34	5.06±0.17	5.42±0.36	6.10±0.39 [†]
SVR, dyne·s·cm ⁻⁵				
P	1,306±132	1,262±72	1,192±98	1,128±48
S	1,332±80	1,251±54	1,260±69	1,082±73 [†]
PAT, ms				
P	146±2	146±3	125±2 [†]	108±2 [†]
S	152±2	158±2 ^{††}	139±3 ^{††}	130±5 ^{††}

*Absolute values (mean±SEM) of HR, MAP, CO, SVR, and PAT at each level of oxygen after pretreatment with placebo (P) or saralasin (S).

[†]Significantly ($p < 0.05$) different from baseline (T_0).

^{††}Significantly different between treatment with saralasin and placebo at that time point.

also noted on both study days, although this reached statistical significance ($p < 0.05$) only during saralasin infusion. HR and MAP were unaffected by hypoxemia on the placebo day, although saralasin infusion resulted in a significant ($p < 0.05$) decrease in MAP at T_3 and T_1 compared to baseline (T_0).

Electrolytes

There was no significant difference in serum sodium or potassium levels at baseline (T_0), between the study days when patients received placebo or saralasin. Serum sodium level was 137.4 ± 0.8 (placebo) vs 138.9 ± 0.8 (saralasin) mmol/L, and serum potassium level was 3.97 ± 0.10 (placebo) vs 3.89 ± 0.08 (saralasin) mmol/L.

RAS Activity

Saralasin infusion resulted in a significant ($p < 0.05$) increase in PRA at T_2 and T_3 compared to baseline (T_0): 5.25 ± 1.36 and 5.60 ± 1.31 vs 3.99 ± 0.55 ng/mL/h, respectively. Hypoxia alone was not associated with a significant change in PRA at T_2 and T_3 compared to baseline (T_0): 3.48 ± 0.65 and 3.29 ± 0.58 vs 3.84 ± 0.77 ng/mL/h, respectively. There was no significant difference in PRA at any of the time points between the 2 study days.

DISCUSSION

Our results demonstrate that the ANG II antagonist saralasin causes pulmonary vasodilatation in the presence of an activated RAS. In this respect, we have shown that absolute MPAP and TPR were significantly lower during hypoxemia after saralasin compared to placebo and similarly that the Δ MPAP and Δ TPR responses from baseline to each level of hypoxemia were also significantly attenuated by saralasin. There is also evidence to suggest that ANG II blockade may attenuate acute hypoxic pulmonary vasoconstriction reflected by a significant reduction in the Δ MPAP and a trend toward a reduction of the Δ TPR response from T_1 to severe hypoxemia (T_3).

Saralasin is a highly soluble and stable ANG II analogue that was developed as an ANG II antagonist.¹⁷ However, saralasin also possesses partial agonist-type activity. It has been demonstrated that saralasin functions as an agonist in the presence of low renin states,^{18,19} whereas elevated levels of PRA and ANG II are associated with antagonism.^{20,21} Consequently, we pretreated our study patients with diuretics to reduce total body sodium concentration and extracellular volume to achieve elevated PRA. We found PRA to be the same after furosemide pretreatment on both study days suggesting comparable degrees of RAS activation. PRA was elevated at baseline in all patients on both days, compared with our own normal reference range

(0.2 to 2.8 ng/mL/h), allowing saralasin to function as an ANG II antagonist rather than agonist. This antagonistic activity is supported by the findings of a significant reduction in MAP at T_1 and T_3 compared to baseline (T_0) when patients received saralasin. We also observed a significant increase in PRA at T_2 and T_3 compared to baseline (T_0), supportive of the antagonistic activity of saralasin in which blockade of ANG II receptors on renin secreting cells would be expected to result in an increase in renin secretion due to inhibition of ANG II mediated negative feedback, occurring at the level of the juxtaglomerular apparatus.

Our results agree with animal studies suggesting a possible role for ANG II in modulating HPV. Berkov⁸ suggested that ANG II was required to facilitate HPV in the saline solution perfused rat lung, and studies in dogs suggest that ANG II infusion augments HPV.⁹ Recently studies in man have shown that ANG II infusion augments HPV although not in a synergistic manner.⁷ The finding that lisinopril pretreatment significantly attenuated acute HPV in normal volunteers suggested that ANG II plays an important role in modulating HPV in normal man.¹¹ Although theoretically this could have been due to increased levels of bradykinin, this agent has not been shown to produce pulmonary vasodilatation in normal humans.²² Saralasin and lisinopril alleviate HPV to similar extents, providing further evidence for a role of ANG II in modulating HPV. Although ANG II undoubtedly has important pressor effects in the pulmonary circulation, studies have suggested that it is not the sole mediator of HPV. McMurty²³ has shown that ANG II is not required for HPV *in vitro*, and studies in man have shown that acute hypoxemia does not increase ANG II levels, although this may not necessarily reflect tissue ANG II activity in the lung.²⁴ In this study, acute hypoxemia had no effect on PRA and ANG II blockade with saralasin only attenuated and did not abolish HPV. This supports the theory that ANG II has a role to play in modulating HPV rather than being the sole mediator of HPV.

How ANG II and hypoxia interact remains unclear, although much interest has surrounded *in vitro* studies showing that pulmonary but not mesenteric arterial myocytes close potassium channels in response to hypoxia.²⁵ This results in membrane depolarization and inward calcium flux through voltage-dependent calcium channels. ANG II is known to increase intracellular calcium through the inositol triphosphate pathway and it has recently been suggested that it may act directly on calcium channels through its receptor.^{26,27} It may be that one subtype of potassium channel acts as a hypoxia sensor in the pulmonary vasculature and ANG II may modulate acute HPV via its effects on calcium flux or on this potassium channel via mem-

brane voltage changes, such that in the presence of ANG II blockade, the hypoxic signal, with respect to pulmonary vasoconstriction, is reduced.

We have used Doppler echocardiography to measure hemodynamic changes in the pulmonary circulation. These noninvasive techniques have been shown to be reproducible¹⁰ and a good correlation between Doppler PAT and MPAP as measured by right heart catheter is well established.^{15,28-30} We looked at two measures of pulmonary vasoconstriction; changes in MPAP and TPR. A possible limitation of this method is that the use of TPR does not account for any changes in the postcapillary vascular bed, as assessed by pulmonary capillary wedge pressure (PCWP). In this respect, we believe it is unethical to insert Swan-Ganz catheters into normal volunteers for research purposes. However, we believe this extra information is not essential. It is known from previous work that hypoxemia has no significant effects on PCWP, suggesting that changes in TPR are reflective of changes in true pulmonary vascular resistance in precapillary arterioles during hypoxia.³¹ To our knowledge, there is no information available regarding long-term dosing with diuretics in normal volunteers; the only study available looked at the acute hemodynamic consequences of IV ethacrynic acid, which showed a reduction in PCWP.³² However, it would be difficult to extrapolate these findings to long-term dosing, and although long-term diuretic therapy may affect PCWP in normal volunteers, it is important to note that patients were exposed to both hypoxia and pretreatment with furosemide on both study days, suggesting that these stimuli are unlikely to be responsible for the changes observed between study days. With respect to saralasin, its infusion has not been shown to affect PCWP.³³ We believe, therefore, that the observed changes in TPR are a true reflection of changes in pulmonary vascular tone.

We have shown for the first time (to our knowledge) in man that ANG II blockade with a specific competitive ANG II antagonist causes pulmonary vasodilatation and alleviates acute HPV in patients with an activated RAS. This suggests the possibility that ANG II is a modulator of acute HPV in normal man. Although one must be careful when extrapolating results from normal volunteer studies, the ability to cause pulmonary vasodilatation and attenuate acute HPV, and therefore the stimulus for pulmonary hypertension in cor pulmonale, suggests that ANG II antagonists may have a role to play in chronic hypoxic lung disease either to prevent or treat the cardiopulmonary consequences of chronic hypoxemia. The availability of orally active ANG II antagonists such as losartan may therefore provide a novel therapeutic avenue for this patient group.

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Acute hypoxic pulmonary vasoconstriction in man is attenuated by type I angiotensin II receptor blockade

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Abstract

Objectives: We examined the hypothesis that angiotensin II (ANG II) is a modulator of acute hypoxic pulmonary vasoconstriction (HPV) by looking at the effect of losartan, a selective type 1 ANG II receptor antagonist, on acute HPV in man. **Methods:** Ten normal volunteers were studied on two separate days. They either received pre-treatment with losartan 25, 50, 100, 100 mg respectively on four consecutive days or matched placebo. They were then rendered hypoxaemic, by breathing an N₂/O₂ mixture for 20 min to achieve an SaO₂ of 85–90% adjusted for a further 20 min to achieve an SaO₂ of 75–80%. Pulsed wave Doppler echocardiography was used to measure mean pulmonary artery pressure (MPAP), cardiac output and hence pulmonary vascular resistance (PVR). **Results:** Baseline MPAP and PVR (during normoxaemia) were unaffected by losartan pre-treatment compared with placebo. However, losartan significantly reduced MPAP at both levels of hypoxaemia compared with placebo: 14.7 ± 0.7 vs 19.0 ± 0.7 mmHg at an SaO₂ 85–90% ($P < 0.01$) and 20.0 ± 0.7 vs 25.7 ± 0.8 mmHg at an SaO₂ 75–80% ($P < 0.05$) respectively. Similarly losartan significantly reduced PVR compared to placebo: 191 ± 9 vs 246 ± 10 dyne \cdot s \cdot cm⁻⁵ at an SaO₂ 85–90% ($P < 0.005$) and 233 ± 12 vs 293 ± 18 dyne \cdot s \cdot cm⁻⁵ at an SaO₂ 75–80% ($P < 0.05$), respectively. Pre-treatment with losartan, however, had no significant effect on systemic vascular resistance although losartan compared to placebo resulted in a significant ($P < 0.05$) reduction in mean arterial pressure at an SaO₂ 75–80%: 78 ± 2 vs 87 ± 2 mmHg. **Conclusions:** Losartan had no effect on baseline pulmonary haemodynamics but significantly attenuated acute hypoxic pulmonary vasoconstriction, suggesting that angiotensin II plays a role in modulating this response in man via its effects on the type 1 angiotensin II receptor.

Keywords: Hypoxia; Pulmonary artery; RAAS; Angiotensin II; Losartan

1. Introduction

Hypoxic pulmonary vasoconstriction (HPV) is an important homeostatic mechanism allowing blood to be diverted from areas of alveolar hypoxia. Although this undoubtedly has beneficial effects, chronic hypoxia results in a continued stimulus to pulmonary vasoconstriction with consequent vascular remodelling and over time the development of cor pulmonale [1–3]. Interestingly, patients with cor pulmonale have activation of the renin angiotensin system (RAS) [4–6], with elevated levels of angiotensin II (ANG II), itself a potent direct pulmonary vasoconstrictor. Whilst in vivo studies have shown ANG II to be a pressor agent in the pulmonary circulation [7] there is conflicting evidence as to whether ANG II is involved in a facilitatory capacity in modulating HPV [8,9].

There has been much research into pharmacological manipulation of the hypoxic pulmonary vasoconstrictor

response although concern surrounds worsening of hypoxaemia by the use of vasodilators which could theoretically have detrimental effects on ventilation perfusion matching. Interestingly, the ANG II antagonist saralasin improved systemic oxygenation presumably by redistributing pulmonary blood flow and improving ventilation perfusion matching [10]. ACE inhibitors have been shown to attenuate the development of pulmonary hypertension in chronically hypoxic rats [11]. A recent study in humans has shown that ACE inhibition attenuated acute HPV [12]. However, this cannot solely be attributed to a reduction in ANG II levels as ACE inhibitors affect many other systems, in particular the kinins and prostanoids.

The purpose of this study, therefore, was to elucidate the role of ANG II and in particular the type 1 ANG II (AT₁) receptor in modulating acute HPV by competitive inhibition with losartan-potassium (DuP 753), a selective orally active specific AT₁ receptor antagonist which shows

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no affinity for other hormonal receptors and at a functional level, excepting ANG II, does not alter the contractile response to a variety of pressor stimuli [13]. Although Jaiswal et al. [14] reported that *in vitro* losartan stimulates prostacyclin release, this finding has not been reproduced by other workers [15,16].

2. Methods

2.1. Subjects

Ten healthy male volunteers, age (mean \pm s.e.m.) 26 ± 5 years, were studied on two separate occasions. There was no abnormality present on clinical history, examination, 12 lead ECG, echocardiography, biochemical or haematological screening. Informed written consent to the study protocol, previously approved by the Tayside Committee for Medical Research Ethics, was obtained.

2.2. Study protocol

Subjects attended the laboratory at the same time of the day on two separate occasions, at least one week apart. Subjects were pre-treated with four daily doses of losartan (25 mg on day 1, 50 mg on day 2, 100 mg on days 3, 4) or matched placebo in random order. They attended the laboratory 3 h after receiving their final dose such that the drug would be studied at maximal effect [17]. An intravenous cannula was inserted into the left forearm for blood sampling. Subjects then rested supine for at least 30 min to obtain stable resting haemodynamics (T_0). They were then rendered hypoxaemic by breathing a variable mixture of oxygen and nitrogen which rendered arterial oxygen saturation 85–90% (T_1) for 20 min then adjusted for a further 20 min to achieve an arterial oxygen saturation of 75–80% (T_2). The hypoxic gas mixture was produced from separate cylinders of nitrogen and oxygen fitted with variable flow valves. Gases were mixed in a 25 litre Douglas bag, from which the subjects breathed through a mouth-piece connected by a series of one-way valves, while wearing an occlusive nose clip. Measurements of pulmonary and systemic haemodynamic variables, and venous blood samples for plasma renin activity (PRA) and electrolyte assays, were taken at T_0 , T_1 , and T_2 .

2.3. Measurements

2.3.1.1. (a) Oxygenation. Arterial blood oxygen saturation was continuously monitored by transcutaneous oximetry (CSI 503, Criticare Systems Inc, Waukesha, WI, USA).

2.3.1.2. (b) Haemodynamics. Heart rate (HR) was recorded on an electrocardiograph trace and an average rate over 1 min was obtained. Mean arterial blood pressure (MAP) was measured by semi-automatic sphygmomanometer (Vital Signs Monitor, Critikon, Tampa, FL, USA). Pulmonary acceleration time (PAT) in milliseconds was measured as previously described [18], from pulmonary arterial flow by pulsed-wave Doppler echocardiography (Vingmed SD50, Vingmed Sound, Horten, Norway) from the left 3rd/4th intercostal space. The mean of three consistent

waveforms at each time point was used for the purpose of analysis. Mean pulmonary artery pressure (MPAP) in mmHg was calculated as $MPAP = 73 - (0.42 \times PAT)$ [19]. Aortic cross-sectional area (CSA) was measured by M-mode echocardiography (Vingmed SD50). The aortic systolic velocity integral (SVI) was measured by on-line computer assisted determination using pulsed-wave Doppler echocardiography of ascending aortic blood flow from the suprasternal notch. On-line calculations of stroke volume ($SV = SVI \times CSA$) and cardiac output (CO) as the product of SV and HR were also made. Total pulmonary vascular resistance (PVR) was calculated as: $PVR = MPAP/CO \times 80 \text{ dyne} \cdot \text{s} \cdot \text{cm}^{-5}$. We have previously shown the short-term coefficients of variability for measurement of PAT and SVI in our hands to be 1.7% and 1.2% respectively [18].

2.3.1.3. (c) Electrolytes. Samples for serum electrolytes were collected in chilled lithium-heparin tubes and were centrifuged at 4°C immediately and separated plasma was stored at –20°C until measured in one batch at the end of the study using an internal caesium standard flame photometer (Instrumentation Laboratory, Milan, Italy).

2.3.1.4. (d) RAS activity. Samples for measurement of plasma renin activity (PRA) were collected into chilled EDTA tubes. They were centrifuged at 4°C immediately and separated plasma was stored at –20°C until assayed in one batch at the end of the study. PRA was assayed using commercially available RIA kits (Sorin Biomedica, Saluggia, Italy) which assayed PRA by measurement of amount of angiotensin I generated per hour. The intra-assay coefficient of variation for analysis of PRA was 7.6%.

2.4. Data analysis

Comparison of values between study days was made by multifactorial analysis of variance (MANOVA) [20]. Comparisons between serial time points on the same study day were made using Duncan's multiple range test. A probability value of $P < 0.05$ (two-tailed) was considered to be statistically significant. Data are presented in the text, tables and figures as means and s.e.m.

3. Results

3.1. Pulmonary haemodynamics

There was no significant difference in absolute values of MPAP or PVR at baseline during normoxaemia (T_0). Losartan compared to placebo resulted in a significant ($P < 0.01$) reduction in MPAP at an SaO_2 of 85–90% (T_1): mean difference 4.3 mmHg (95% CI 1.6–7.0) and a significant ($P < 0.05$) difference at an SaO_2 of 75–80% (T_2): mean difference 5.6 mmHg (95% CI 2.8–8.5), respectively (Fig. 1A). Likewise losartan compared to placebo resulted in a significant ($P < 0.005$) reduction in PVR at T_1 : mean difference 55 $\text{dyne} \cdot \text{s} \cdot \text{cm}^{-5}$ (95% CI 24–86) and a significant ($P < 0.05$) reduction at T_2 : mean difference 60 $\text{dyne} \cdot \text{s} \cdot \text{cm}^{-5}$ (95% CI 9–110), respectively (Fig. 1B). Hypoxaemia was associated with a signifi-

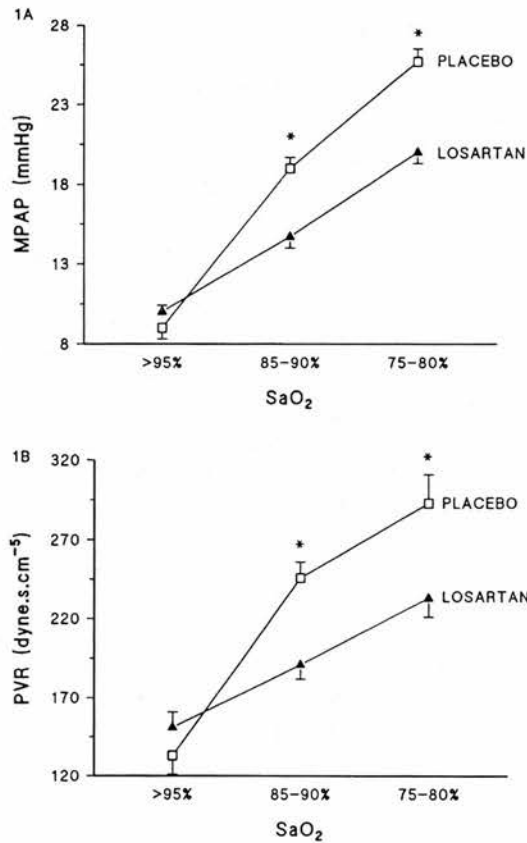


Fig. 1. (A) Absolute Doppler mean pulmonary artery pressure (MPAP) measured at (a) baseline during normoxaemia, (b) at an SaO₂ 85–90% and (c) at an SaO₂ 75–80%. Asterix represents a significant ($P < 0.05$) difference between placebo and losartan at each time point. (B) Absolute pulmonary vascular resistance (PVR) measured at (a) baseline during normoxaemia, (b) at an SaO₂ 85–90% and (c) at an SaO₂ 75–80%. Asterix represents a significant ($P < 0.05$) difference between placebo and losartan at each time point.

icant ($P < 0.001$) increase in MPAP and PVR at T₁ and T₂ compared to baseline (T₀) on both study days.

In terms of change in MPAP (Δ MPAP) from T₀ to T₁ and T₀ to T₂, the Δ MPAP response induced by hypoxaemia was significantly ($P < 0.01$) attenuated by losartan compared with placebo (Fig. 2A): mean difference 5.2 mmHg (95% CI 2.0–8.4) and 6.6 mmHg (95% CI 3.3–9.9) respectively. Likewise the Δ PVR response induced by hypoxaemia from T₀ to T₁ and T₀ to T₂ was significantly ($P < 0.01$) attenuated by losartan compared with placebo

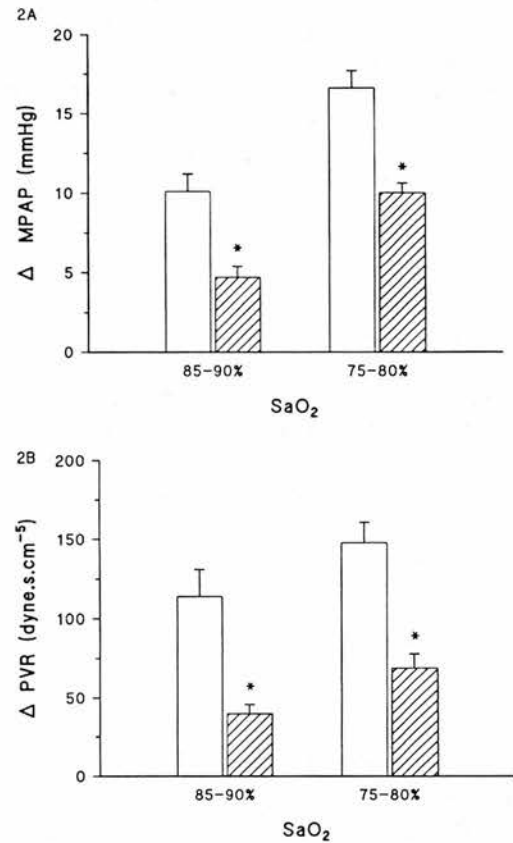


Fig. 2. (A) Change in mean pulmonary artery pressure (Δ MPAP) from baseline induced by hypoxaemia (a) at an SaO₂ of 85–90% (i.e. T₀ to T₁) and (b) at an SaO₂ of 75–80% (i.e. T₁ to T₂). The Δ MPAP represents the mean of the individual changes. Asterix denotes that the hypoxic pulmonary vasoconstrictor response was significantly ($P < 0.01$) attenuated by losartan (hatched bars) compared with placebo (clear bars) at an SaO₂ of 85–90% and at an SaO₂ of 75–80%. (B) Change in pulmonary vascular resistance (Δ PVR) induced by hypoxaemia (a) at an SaO₂ of 85–90% and (b) at an SaO₂ of 75–80%, compared to baseline. The Δ PVR represents the mean of the individual changes. Asterix denotes that the hypoxic pulmonary vasoconstrictor response in man was significantly ($P < 0.01$) attenuated by losartan (hatched bars) compared with placebo (clear bars) at an SaO₂ of 85–90% and at an SaO₂ of 75–80%.

(Fig. 2B): mean difference 70 dyne \cdot s \cdot cm⁻⁵ (95% CI 26–152) and 78 dyne \cdot s \cdot cm⁻⁵ (95% CI 29–127).

3.2. Systemic haemodynamics

There was no significant difference between HR, CO and SVR at each time point between the two study days

Table 1
Systemic haemodynamics

		SaO ₂ > 95% (T ₀)	SaO ₂ 85–90% (T ₁)	SaO ₂ 75–80% (T ₂)
HR (bpm)	P	58 \pm 2	64 \pm 2	70 \pm 3*
	L	61 \pm 2	67 \pm 3	73 \pm 3*
MAP (mmHg)	P	85 \pm 3	85 \pm 2	87 \pm 2
	L	80 \pm 2	80 \pm 2	78 \pm 2 ⁺
CO (l/min)	P	5.52 \pm 0.17	6.17 \pm 0.11	7.18 \pm 0.37*
	L	5.39 \pm 0.24	6.22 \pm 0.33	6.96 \pm 0.36*
SVR (dyne \cdot s \cdot cm ⁻⁵)	P	1232 \pm 42	1098 \pm 33	994 \pm 65*
	L	1209 \pm 39	1060 \pm 62	909 \pm 43*

* Significantly ($P < 0.05$) different from SaO₂ > 95%.

⁺ Significantly ($P < 0.05$) different from placebo at the same time point.

Values are shown as means \pm standard error of the mean after prior treatment with losartan or placebo at each level of oxygenation.

HR = heart rate; MAP = mean arterial pressure; CO = cardiac output; SVR = systemic vascular resistance.

P = placebo; L = losartan.

Table 2

Serum electrolytes and plasma renin activity

		SaO ₂ > 95% (T ₁)	SaO ₂ 85–90% (T ₁)	SaO ₂ 75–80% (T ₁)
Na ⁺ (mmol/l)	P	139.1 ± 0.4	139.3 ± 0.	139.3 ± 0.4
	L	139.2 ± 0.4	139.7 ± 0.3	139.3 ± 0.3
K ⁺ (mmol/l)	P	4.08 ± 0.09	4.09 ± 0.12	4.01 ± 0.10
	L	4.15 ± 0.06	4.11 ± 0.06	4.05 ± 0.07
PRA (pmol/l·h ⁻¹)	P	1.00 ± 0.14	1.08 ± 0.19	0.84 ± 0.12
	L	7.48 ± 1.42 ⁺	6.62 ± 1.18 ⁺	6.46 ± 1.02 ⁺

⁺ Significantly ($P < 0.01$) different from placebo at the same time point.

Values are shown as means ± standard error of the mean after prior treatment with losartan or placebo at each level of oxygenation.

Na⁺ = serum sodium; K⁺ = serum potassium; PRA = plasma renin activity.

P = placebo; L = losartan.

although severe hypoxaemia (at T₂) resulted in a significant ($P < 0.01$) increase in CO and HR and a significant decrease in SVR on both study days. There was no significant difference between study days with respect to MAP at baseline (T₀) or T₁, although in the presence of severe hypoxaemia (T₂), MAP was significantly ($P < 0.05$) lower following pre-treatment with losartan compared with placebo: mean difference 9 mmHg (95% CI 2–16). Hypoxaemia had no significant effects on MAP on either study day (Table 1).

3.3. Electrolytes

There was no significant difference in serum sodium or potassium following treatment with losartan or placebo at any time point. Similarly, hypoxia had no significant effects on serum sodium or potassium in comparison with baseline (T₀) on either study day (Table 2).

3.4. RAS activity

Pre-treatment with losartan resulted in a significant ($P < 0.01$) increase in PRA compared to placebo at baseline (T₀) at an SaO₂ of 85–90% (T₁) and at an SaO₂ of 75–80% (T₂). Hypoxia did not significantly affect PRA on either study day in comparison with baseline (Table 2).

4. Discussion

We have demonstrated that the selective type 1 ANG II blocker losartan attenuates acute hypoxic pulmonary vasoconstriction in man. In this respect both absolute MPAP and absolute PVR were significantly lower during hypoxia in those pre-treated with losartan compared to placebo. In addition, to correct for any possible confounding baseline effects, we have also shown that the delta-MPAP and delta-PVR responses to each level of hypoxia were significantly attenuated by losartan. This supports the hypothesis that ANG II may modulate the acute HPV response in man via its effects on the AT₁ receptor.

Losartan is a potent, orally active drug which selectively blocks the AT₁ receptor [13] and as such provides us with a specific tool for elucidating the role of ANG II in HPV. It has been shown to produce concentration-dependent inhibition of angiotensin II induced vasoconstriction in vitro and in vivo [17,21]. Studies using radioligand membrane binding and autoradiography techniques have

shown heterogenicity of ANG II receptors in a variety of different tissues [13]; however, the relative importance of the AT₁ and AT₂ receptor subtypes with respect to pulmonary pressor effects has not been previously documented in man. Our results support the hypothesis that ANG II has its effect primarily via the AT₁ receptor since blockade of the AT₁ receptor significantly attenuated this response. We also observed a significant increase in PRA after treatment with losartan during both normoxaemia and hypoxaemia. This is consistent with blockade of ANG II receptors in the juxtaglomerular apparatus resulting in renin secretion due to inhibition of ANG II mediated negative feedback. Indeed, juxtaglomerular cell hypertrophy and hyperplasia has been shown to occur as a consequence of losartan therapy in rhesus monkeys [22]. In contrast, if the AT₂ receptor was as important in mediating the acute HPV response then one would have expected a nullification or a paradoxical increase in response after treatment with losartan compared to placebo occurring as a result of elevated ANG II levels. Although the pulmonary and systemic vascular beds may respond differently to the same stimulus, these results agree with findings in the systemic circulation which have shown that none of the established cardiovascular effects of ANG II can be attributed to the AT₂ receptor [13].

Our findings are consistent with those in animal studies suggesting an important role for ANG II in modulating HPV. Berkov suggested that ANG II was required to facilitate HPV in the saline perfused rat lung [8] and ANG II infusion has been shown to augment HPV in dogs [23]. Studies in man have shown that ANG II infusion augments HPV although not synergistically [24]. Interestingly, the finding that pre-treatment with lisinopril attenuated acute HPV in normal volunteers suggested an important role for ANG II in modulating HPV in normal man [17]. This could theoretically have been due to increased bradykinin levels although bradykinin infusion did not produce pulmonary vasodilation in normal volunteers [25]. In this study we have shown that losartan attenuates HPV to a similar degree as lisinopril, further implicating ANG II as having an important role in modulating HPV. Although ANG II undoubtedly has important pressor effects in the pulmonary circulation, it is unlikely to be the sole mediator of HPV. McMurty has shown that ANG II is not required to produce HPV in vitro [9] and studies in man have shown that acute hypoxaemia does not increase ANG II levels [26]. This is consistent with the present study where

we have shown that acute hypoxaemia had no significant effect on PRA and that ANG II blockade with losartan only attenuated rather than abolished HPV. This suggests that, rather than being the sole mediator of HPV, ANG II has an important role in modulating this response.

The mechanism whereby ANG II and hypoxaemia interact remains unclear, although in vitro studies have shown that pulmonary but not mesenteric vascular smooth muscle cells react to hypoxia by closing potassium channels [27]. The resultant membrane depolarisation results in an increase in intracellular calcium as a result of calcium influx through voltage-dependent calcium channels and consequently smooth muscle contraction. ANG II is known to increase intracellular calcium due to increased inositol triphosphate production [28] and it has recently been suggested that it may act directly on calcium channels [29]. It may be that one subtype of potassium channel acts as a hypoxia sensor in the pulmonary vasculature and that ANG II may modulate acute HPV via its effect on calcium flux or this potassium channel via membrane voltage changes, such that in the absence of ANG II the hypoxic signal, with respect to pulmonary vasoconstriction, is reduced.

With respect to methodology, we have used Doppler echocardiography to measure haemodynamic changes in the pulmonary circulation. These non-invasive techniques have been shown to be highly reproducible [18] and the close correlation between Doppler PAT and MPAP as measured by right heart catheter, is well established [19,30,31]. We looked at two measures of pulmonary vasoconstriction; changes in MPAP and PVR. A possible limitation of this methodology is that the use of total PVR does not account for any changes in the post-capillary vascular bed, as conventionally assessed by pulmonary capillary wedge pressure (PCWP). In this respect we feel it is unethical to insert Swan-Ganz catheters into normal volunteers for research purposes particularly as volunteers would have to be restudied on different days. The extra information this would give us is, however, not essential. Both groups were exposed to hypoxia and it is known from previous work that hypoxaemia does not affect PCWP and so effects on total PVR are reflective of changes in true PVR in pre-capillary arterioles during hypoxia [32]. With respect to ANG II blockade, an acute dosing study looking at the haemodynamic effects of losartan has shown that it does not affect PCWP [33]. We believe therefore, that the observed changes in total PVR are a true reflection of changes in pulmonary vascular tone.

Thus, we have shown for the first time in man that ANG II blockade with a type 1 ANG II antagonist attenuates acute HPV. This suggests that ANG II acts as an important modulator of acute HPV in healthy humans via its effects on the AT₁ receptor. One must be cautious when extrapolating from normal volunteer studies to the clinical situation. However, the ability of losartan to attenuate HPV, the initial stimulus for pulmonary vascular remodelling, suggests that it may delay or prevent progression from chronic hypoxic lung disease to cor pulmonale. Losartan may also have a role to play in the treatment of cor pulmonale where vasoreactivity is still present. This benefit may not only result from the interaction between

ANG II and hypoxia with respect to the pulmonary vascular bed, but also as a consequence of blockade of the direct pulmonary pressor effects of ANG II, particularly in patients on oxygen therapy, where ANG II would be predicted to exhibit proportionately greater pressor effects [24].

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Haemodynamic and endocrine effects of type 1 angiotensin II receptor blockade in patients with hypoxaemic cor pulmonale

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Abstract

Objectives: Angiotensin II (ANG II) is known to be a potent vasoconstrictor agent in the pulmonary circulation. Furthermore, type 1 ANG II receptor blockade with losartan attenuates acute hypoxic pulmonary vasoconstriction in normal subjects. The aim of this study was therefore to evaluate the haemodynamic and endocrine sequelae of type 1 ANG II receptor blockade in patients with hypoxaemic cor pulmonale. **Methods:** Nine patients with chronic obstructive pulmonary disease (COPD) age 67 ± 3 years with pulmonary hypertension and normal left ventricular systolic function were studied on two separate occasions in a double-blind, placebo-controlled, crossover study. They were randomised to receive either 50 mg of oral losartan or matched placebo. Pulsed wave Doppler echocardiography was used to measure cardiac output (CO), mean pulmonary artery pressure (MPAP) and hence systemic vascular resistance (SVR) and total pulmonary vascular resistance (TPR). Haemodynamic measurements and venous blood samples were taken at baseline and after 2 and 4 h. **Results:** Maximal effects were observed at 4 h where losartan compared to placebo resulted in a significant reduction in both MPAP (28.6 ± 2.0 vs 32.4 ± 1.5 mmHg) and TPR (428 ± 40 vs 510 ± 40 dyn \cdot s \cdot cm⁻⁵), respectively. Similarly losartan compared to placebo resulted in a significant reduction in MAP (87 ± 4.5 vs 93 ± 3.2 mmHg) and SVR (1293 ± 94 vs 1462 ± 112 dyn \cdot s \cdot cm⁻⁵), and significantly increased CO (5.58 ± 0.43 vs 5.31 ± 0.42 l/min). In addition, plasma aldosterone was significantly lower after treatment with losartan compared to placebo: 76 ± 23 vs 164 ± 43 pg/ml respectively. **Conclusions:** Thus, selective type 1 ANG II receptor blockade appears to have beneficial pulmonary and endocrine effects, suggesting a possible therapeutic role in the management of hypoxaemic cor pulmonale.

Keywords: Angiotensin II; Losartan; Angiotensin receptor; Pulmonary hypertension; Human

1. Introduction

Acute hypoxic pulmonary vasoconstriction (HPV) is an important phenomenon present in a wide variety of different animal species allowing blood to be diverted from areas of alveolar hypoxia, so maintaining ventilation perfusion matching. Although this undoubtedly has beneficial effects, it can also have deleterious effects. In particular, in chronic hypoxic lung disease, hypoxaemia provides a sustained stimulus to pulmonary vasoconstriction, resulting in an elevation of mean pulmonary artery pressure [1], vascular remodelling [2] and over time the development of cor pulmonale and consequently a poor prognosis [3,4].

The benefits of treating pulmonary hypertension in the context of chronic hypoxic lung disease are unknown. Oxygen therapy has been shown to reduce mean pulmonary artery pressure acutely [5], and reduce mortality in patients with hypoxaemic cor pulmonale [3,4]. Whether this is primarily due to improved systemic oxygenation or a reduction in right ventricular afterload is not known. Nevertheless, therapy that could either prevent or treat pulmonary hypertension in these patients could conceivably reduce both mortality and morbidity and have additional benefits to oxygen therapy.

In the context of pulmonary hypertension, the role of vasoactive peptides has been extensively investigated [6]. It is known that angiotensin II (ANG II) is a potent

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pulmonary vasoconstrictor in man [7,8] and that patients with hypoxaemic cor pulmonale have activation of the renin–angiotensin–aldosterone system (RAAS) [9,10]. Indeed, ANG II has been shown to promote a growth response in vascular smooth muscle cells [11], suggesting the possibility that, in addition to acting as pressor agent, ANG II could also contribute directly to vascular remodelling. Interestingly angiotensin II converting enzyme inhibitors (ACE inhibitors) have been shown to attenuate the development of pulmonary hypertension in chronically hypoxic rats [12], although there is conflicting evidence from *in vitro* studies as to whether ANG II plays a facilitatory role in modulating HPV [13,14]. In normal humans, however, ACE inhibition with lisinopril [15] and type 1 ANG II receptor blockade with losartan [16] have been shown to attenuate acute HPV. Subsequent studies in patients with hypoxaemic cor pulmonale using ACE inhibitors have shown variable pulmonary and systemic haemodynamic benefit [17–21]. Whether the beneficial haemodynamic effects are a consequence of reducing ANG II levels is unknown since ACE inhibitors also increase levels of bradykinin, a vasodilator.

We have therefore evaluated for the first time the effects of selective type 1 ANG II receptor blockade with losartan, in patients with hypoxaemic cor pulmonale.

2. Methods

2.1. Subjects

Nine patients (6 male, 3 female), with clinically stable cor pulmonale secondary to hypoxaemic cor pulmonale (mean age \pm s.e.m. 67 ± 3 years), were included in the study after attending a screening visit to assess inclusion criteria and characterise the study population. All had spirometric evidence of obstructive airways disease ($FEV_1/FVC < 70\%$) and arterial hypoxaemia whilst breathing air ($PaO_2 < 8.5$ kPa) and had or gave a history of having peripheral oedema. On echocardiography subjects were required to be in sinus rhythm, have normal left ventricular function, no evidence of valvular heart disease and a resting mean pulmonary artery pressure, whilst

breathing room air, of at least 25 mmHg. In addition, all subjects had evidence of reversible, dynamic pulmonary vasoconstriction as assessed by $\geq 10\%$ fall in MPAP on breathing 60% oxygen for 30 min. All subjects were taking inhaled bronchodilators (beta-agonist $n = 9$; anticholinergic $n = 6$) and inhaled steroids, five patients were taking oral loop diuretics and 6 patients used domiciliary oxygen for at least 15 h per day. Medications were unchanged throughout the study period. The summary demographic data for this patient group are given in Table 1.

2.2. Study protocol

All subjects gave informed written consent to the study protocol previously approved by the Tayside Committee for Medical Research Ethics and conforming with the principles outlined in the Declaration of Helsinki. Subjects were studied at the same time of the day on 2 separate occasions at least 1 week apart in a randomised, double-blind placebo-controlled, cross-over design. Patients taking regular diuretics were asked to omit their morning dose of diuretic on each visit. On each study day, an intravenous cannula was sited in the right forearm for venous blood sampling. Subjects then remained semi-recumbent throughout and were studied whilst breathing room air. Patients then received either 50 mg of oral losartan potassium (Merck Sharp and Dohme Ltd, Hertfordshire, UK) or placebo. Haemodynamic parameters were measured and blood samples were taken at baseline after subjects had rested for at least 30 min to obtain stable resting haemodynamics (T_0), 2 h (T_1) and 4 h (T_2) after administration of either losartan or placebo.

2.3. Measurements

2.3.1. Oxygenation

Arterial blood oxygen saturation was continuously monitored by transcutaneous oximetry (CSI 503, Criticare Systems Inc, Waukesha, WI, USA).

2.3.2. Systemic haemodynamics

Mean arterial blood pressure (MAP) and heart rate (HR) were measured by semi-automatic sphygmomanometer (Vital Signs Monitor, Critikon, Tampa, FL, USA) as the mean of 3 consistent readings. Aortic cross-sectional area (CSA) was measured by M-mode echocardiography (Vingmed SD50). The aortic systolic velocity integral (SVI) was measured by on-line computer-assisted determination using pulsed-wave Doppler echocardiography of ascending aortic blood flow from the suprasternal notch. On-line calculations of stroke volume ($SV = SVI \times CSA$) and cardiac output (CO) as the product of SV and HR were made. Total systemic vascular resistance (SVR) was calculated as: $SVR = MAP/CO \times 80 \text{ dyn} \cdot \text{s} \cdot \text{cm}^{-5}$. Reproducibility of our haemodynamic measurements includ-

Table 1
Patient characteristics

FEV_1 (l)	0.78 ± 0.09
$FEV_1\%$ predicted (%)	30 ± 2
FEV_1/FVC	43 ± 3
PaO_2 (kPa) on air	7.01 ± 0.24
$PaCO_2$ (kPa) on air	6.84 ± 0.28
MPAP (mmHg)	32 ± 2

The above values are expressed as means \pm s.e.m. for respiratory function and pulmonary haemodynamics in the patient group studied. FEV_1 = forced expiratory volume in 1 s; FEV_1/FVC = ratio of FEV_1 to forced vital capacity; MPAP = mean pulmonary artery pressure.

ing Doppler methodology was assessed in patients with cor pulmonale by 3 repeat measurements over a 4 h period at 2 h intervals. This short-term co-efficient of variability for MAP was 8.2%, CO was 9.6% and SVR was 5.3%.

2.3.3. Pulmonary haemodynamics

Pulmonary arterial flow was analysed by pulsed-wave Doppler echocardiography (Vingmed SD50) from the sub-costal position to measure the pulmonary acceleration time (PAT), being the time in milliseconds from the onset of pulmonary flow to peak velocity. A stable pulsed-wave Doppler signal was recorded with the mean of 3 consistent waveforms at each time point used for the purpose of analysis. Mean pulmonary artery pressure (MPAP) in mmHg was then calculated using the regression equation $MPAP = 90 - (0.62 \times PAT)$ as described by Dabestani for pulmonary acceleration times < 110 ms [22].

Fig. 1 shows the correlation in our own hands between measurements of PAT and MPAP made simultaneously using pulsed-wave Doppler echocardiography in patients

undergoing right heart catheterisation after acute admission to a coronary care unit, over a range of pulmonary artery pressures ($r = -0.88$, $y = 164 - 0.88 \times$, $P < 0.0001$, $n = 17$). Using Dabestani's regression equation, $MPAP = 73 - (0.42 \times PAT)$ applicable to this range of pulmonary acceleration times, a close correlation between Doppler and catheter MPAP was also obtained ($r = 0.88$, $y = 0.79 \times + 4.2$, $P < 0.0001$, $n = 17$). Total pulmonary vascular resistance (TPR) was calculated as $TPR = MPAP/CO \times 80 \text{ dyn} \cdot \text{s} \cdot \text{cm}^{-5}$. Reproducibility was assessed as before and the short term co-efficient of variability for PAT was 3.2%, MPAP was 5.8% and TPR was 12.4%.

2.3.4. RAAS activity and plasma creatinine

Samples for measurement of plasma renin activity (PRA) were collected into chilled EDTA tubes and samples for plasma aldosterone were collected in chilled lithium-heparin tubes. They were centrifuged at 4°C immediately and separated plasma was stored at -20°C until assayed in one batch in duplicate at the end of the study. PRA and plasma aldosterone were assayed using commercially available RIA kits (Sorin Biomedica, Saluggia, Italy). PRA was assayed by measurement of amount of ANG I generated per hour. The intra-assay coefficient of variation for analysis of PRA was 6.50% and plasma aldosterone was 8.77%.

2.4. Data analysis

Comparison of values between study days was made by multifactorial analysis of variance (MANOVA) [23] and where there was a significant difference the mean difference and 95% confidence intervals for the mean difference are given (95% CI). Comparisons between serial time points on the same study day were made using Duncan's multiple range test. A probability value of $P < 0.05$ (two-tailed) was considered to be statistically significant. Data are presented as means and s.e.m.

3. Results

3.1. Systemic haemodynamics

There were no significant differences in absolute values of HR, SV, CO, MAP or SVR at baseline between study days. Although there was no significant difference in either MAP or SVR after treatment with losartan or placebo at 2 h, both MAP ($P < 0.05$, mean difference 5.7 mmHg, 95% CI 0.4–10.9) and SVR ($P < 0.01$, mean difference 169 $\text{dyn} \cdot \text{s} \cdot \text{cm}^{-5}$, 95% CI 71–267) were significantly lower 4 h after treatment with losartan compared to placebo (Fig. 2). Although losartan compared to placebo had no significant effects on SV, CO was significantly ($P < 0.05$) higher 4 h after losartan compared to placebo: mean difference

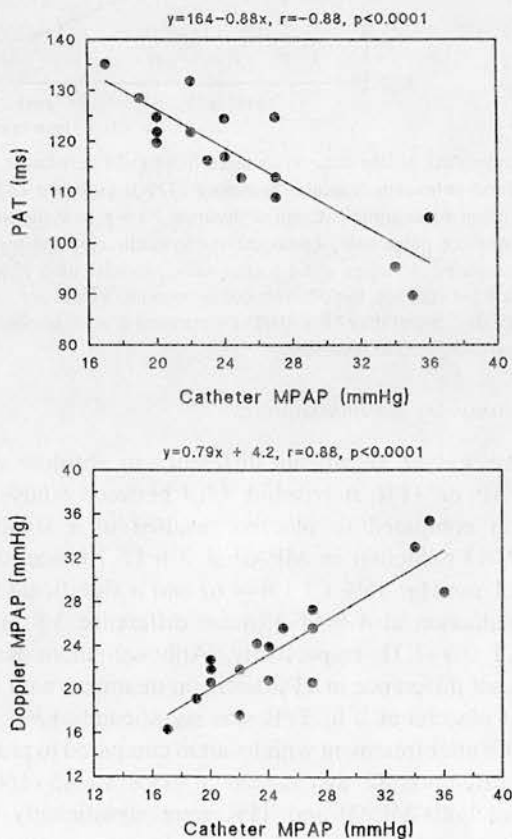


Fig. 1. Validation of Doppler-derived measures of mean pulmonary artery pressure. Upper panel: Data comparing pulmonary acceleration time (PAT) and simultaneously measured mean pulmonary artery pressure (MPAP) during right heart catheterisation in patients with suspected pulmonary hypertension. Lower panel: Data comparing catheter- and Doppler-derived measures of mean pulmonary artery pressure (calculated using Dabestani's regression equation $MPAP = 73 - [0.42 \times PAT]$) during right heart catheterisation in patients with suspected pulmonary hypertension.

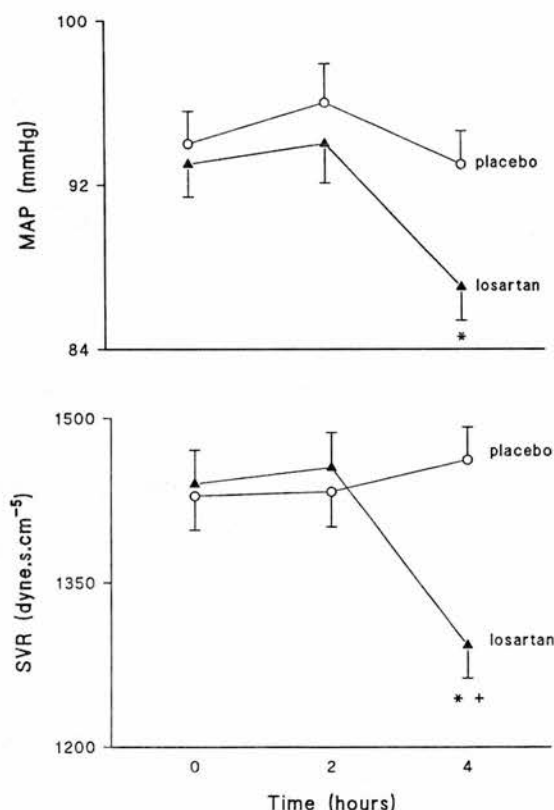


Fig. 2. Systemic haemodynamics. *Upper panel*: Mean arterial blood pressure (MAP) measured at baseline, after 2 h and after 4 h. *Lower panel*: Systemic vascular resistance (SVR) measured at baseline, after 2 h and after 4 h. Treatment with losartan is represented by the bold triangles whereas treatment with placebo is represented by the open circles. Values are given as mean \pm s.e.m. * Represents a significant ($P < 0.05$) difference between placebo and losartan at that time point whereas a + sign represents a significant ($P < 0.05$) difference between that timepoint and baseline.

0.27 l/min (95% CI 0.04–0.50) (Table 2). Fig. 3 shows the changes in SVR 4 h after administration of losartan compared to placebo for individual patients. Whilst there was no significant difference in HR between study days at 4 h, HR was significantly higher 4 h after treatment with losartan compared to baseline (Table 2).

Table 2
Systemic haemodynamics

		T_0	T_1	T_2
CO (l/min)	P	5.47 \pm 0.42	5.56 \pm 0.49	5.31 \pm 0.42
	L	5.26 \pm 0.32	5.37 \pm 0.46	5.58 \pm 0.43 *
SV (ml)	P	79 \pm 7	79 \pm 7	75 \pm 7
	L	76 \pm 6	78 \pm 6	76 \pm 6
HR (bpm)	P	73 \pm 2	73 \pm 2	75 \pm 2
	L	75 \pm 3	73 \pm 3	81 \pm 4 +

Values are given as mean \pm s.e.m. CO = cardiac output; SV = stroke volume; HR = heart rate. P = placebo; L = losartan.

* Significantly ($P < 0.05$) different from placebo at the same time point.

+ Significantly ($P < 0.05$) different from T_0 .

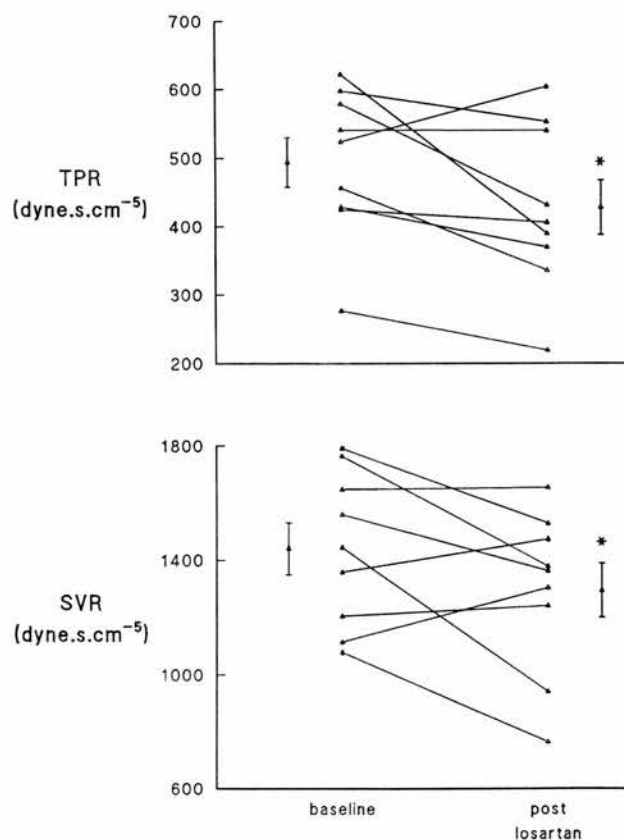


Fig. 3. Individual patient data on changes in vascular resistance. *Upper panel*: Total pulmonary vascular resistance (TPR), measured at baseline and 4 h after acute administration of losartan 50 mg in 9 patients with hypoxaemic cor pulmonale. *Lower panel*: Systemic vascular resistance (SVR), measured at baseline and 4 h after acute administration of losartan 50 mg in 9 patients with hypoxaemic cor pulmonale. Values are given as mean \pm s.e.m. * Significant ($P < 0.05$) difference 4 h after administration of losartan compared to baseline.

3.2. Pulmonary haemodynamics

There was no significant difference in absolute values of MPAP or TPR at baseline (T_0) between study days. Losartan compared to placebo resulted in a significant ($P < 0.01$) reduction in MPAP at 2 h (T_1) (mean difference 3.1 mmHg, 95% CI 1.6–4.6) and a significant ($P < 0.05$) reduction at 4 h (T_2) (mean difference 3.8 mmHg, 95% CI 0.3–7.3), respectively. Although there was no significant difference in TPR between treatment with losartan and placebo at 2 h, TPR was significantly ($P < 0.05$) lower 4 h after treatment with losartan compared to placebo: mean difference 82 dyn \cdot s \cdot cm⁻⁵ (95% CI 15–150). In addition, both MPAP and TPR were significantly ($P < 0.05$) lower 4 h after treatment with losartan compared to baseline (Fig. 4). Fig. 3 shows the changes in TPR 4 h after administration of losartan compared to baseline for individual patients.

3.3. RAAS activity and serum creatinine

There were no significant differences in absolute values of PRA, plasma aldosterone or serum creatinine at baseline

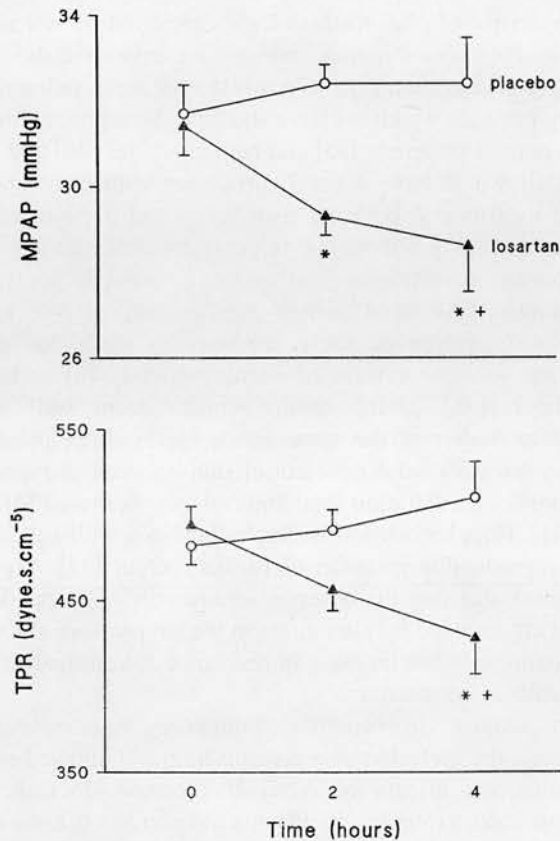


Fig. 4. Pulmonary haemodynamics. Upper panel: Doppler mean pulmonary artery pressure (MPAP) measured at baseline, after 2 h and after 4 h. Lower panel: Total pulmonary vascular resistance (TPR) measured at baseline, after 2 h and after 4 h. Treatment with losartan is represented by the bold triangles whereas treatment with placebo is represented by the open circles. Values are given as mean \pm s.e.m. * Represents a significant ($P < 0.05$) difference between placebo and losartan at that time point whereas a + sign represents a significant ($P < 0.05$) difference between that timepoint and baseline.

between study days. Although there were no significant differences between study days for PRA either at baseline or 2 and 4 h after administration of losartan or placebo, PRA was significantly increased 4 h after administration of losartan compared to baseline. There were significant (P

< 0.05) falls in plasma aldosterone with time on both study, but in addition losartan compared to placebo resulted in a significant ($P < 0.05$) reduction in plasma aldosterone at 4 h (T_2) (mean difference 89 pg/ml, 95% CI 19–159). There were no significant differences in plasma creatinine at baseline (T_0), T_1 or T_2 between study days or with time on either study day (Table 3).

3.4. Oxygen saturation

There were no significant differences in oxygen saturation between days on which patients received placebo or losartan at baseline (T_0) (91.4 ± 1.2 vs $91.1 \pm 1.1\%$), at T_1 (90.7 ± 1.1 vs $90.7 \pm 1.1\%$), or at T_2 (90.7 ± 1.2 vs $90.8 \pm 1.1\%$), respectively, or with time on either study day.

4. Discussion

We have demonstrated for the first time that the selective type 1 ANG II receptor blocker, losartan, has beneficial pulmonary haemodynamic effects in hypoxaemic cor pulmonale without altering oxygen saturation. In this respect both MPAP and TPR were significantly lower after treatment with losartan compared to placebo, although this effect was of small magnitude and of uncertain clinical relevance. In addition, type 1 ANG II receptor blockade appears to have beneficial endocrine effects reflected by significantly lower levels of plasma aldosterone after treatment with losartan compared to placebo. We also noted a significant reduction in both MAP and SVR as well as a small but significant increase in cardiac output after treatment with losartan. This suggests that ANG II blockade may have a therapeutic role in the management of hypoxaemic cor pulmonale.

Losartan a potent, orally active type 1 ANG II receptor blocker (AT_1) has provided us with a tool for elucidating the role of ANG II in the pulmonary circulation in man. It is a selective orally active selective AT_1 receptor antagonist which shows no affinity for other hormonal receptors and at a functional level excepting ANG II it does not alter contractile response to a variety of different stimuli [24] and has been shown to produce concentration-dependent inhibition of ANG-II-induced vasoconstriction in vitro and in vivo [25,26]. Losartan undergoes an important first-pass effect and is extensively transformed to its active metabolite, EXP 3174, reaching peak concentrations between 2 and 4 h after oral administration of losartan [27]. Early studies in normal volunteers have confirmed antagonism of pressor responses to exogenous administration of ANG I and ANG II with approximately 60–70% inhibition of control responses at maximum effect (3 h) [25,28]. Although the interaction of losartan with the eicosanoid system has been observed in animals with high concentra-

Table 3
Renin-angiotensin-aldosterone system activity and serum creatinine

		T_0	T_1	T_2
PRA (ng/ml/hr)	P	4.21 ± 1.50	4.97 ± 1.92	4.40 ± 1.38
	L	4.54 ± 1.62	5.66 ± 1.97	9.46 ± 2.56 +
Aldosterone (pg/ml)	P	238 ± 50	187 ± 45 +	164 ± 43 +
	L	179 ± 43	142 ± 45	76 ± 23 * +
Creatinine (μ mol/l)	P	106 ± 11	102 ± 11	101 ± 9
	L	101 ± 10	99 ± 11	99 ± 9

Values are given as mean \pm s.e.m. PRA = plasma renin activity.

* Significantly ($P < 0.05$) different from placebo at the same time point.

+ Significantly ($P < 0.05$) different from T_0 .

tions of the drug by some workers [29,30] it seems unlikely that this accounts for the observed effects to a significant degree. Indeed, conflicting results surround evidence suggesting that losartan stimulates prostacyclin synthesis [29,31,32].

We examined the acute haemodynamic effects of a single dose of oral losartan. Our patients had activation of the RAAS system reflected by elevated levels of PRA at baseline compared with our own normal reference range (0.2–2.8 ng/ml/h). We also observed a significant increase in PRA 4 h after administration of losartan compared to baseline, consistent with blockade of the type 1 ANG II receptor in the juxtaglomerular apparatus, resulting in renin secretion due to inhibition of ANG II mediated negative feedback.

In terms of both systemic and pulmonary haemodynamic changes we observed maximal effect at 4 h. Whether the peak effect may have occurred after 4 h or if larger doses of losartan would achieve greater pulmonary haemodynamic benefit or whether this would be limited by systemic haemodynamic effects is not answered by this study. Interestingly, acute dosing studies in patients with congestive cardiac failure have shown no greater systemic haemodynamic effects with doses of losartan of greater than 25 mg of losartan and noted falls in MAP and SVR after 2 h with peak effect observed between 4 and 12 h and an effect still seen at 24 h [33]. This study does however suggest that ANG II contributes to the maintenance of both pulmonary and systemic vascular tone in patients with hypoxaemic cor pulmonale. Although ANG II has been shown to have pressor effects in man [7,8], studies using ACE inhibitors in patients with hypoxaemic cor pulmonale have not consistently shown a pulmonary haemodynamic benefit, possibly as a consequence of inclusion criteria [17–21]. We have, however, specifically excluded patients who did not have demonstrable pulmonary vascular reactivity as assessed by the absence of a significant fall in mean pulmonary artery pressure in response to acute oxygen administration. In this respect we feel it is important that patients who are included in vasodilator trials have at least some dynamic component to their pulmonary hypertension, a situation analogous to studying bronchodilator therapy, where the degree of airway reversibility is established before evaluating the impact of therapy.

We observed a small but significant increase in cardiac output after treatment with losartan compared to placebo, and although HR was not significantly different between study days, losartan significantly increased HR compared to baseline. Although no significant increase in heart rate has been observed in acute dosing studies with losartan in patients with congestive cardiac failure, it is interesting that an increase in HR was noted in studies performed in normal volunteers pre-treated with oral frusemide [34]. This could possibly represent a reflex vagal withdrawal response to an acute reduction in afterload or a direct effect of losartan on the autonomic nervous system.

In terms of the methodology employed to measure pulmonary haemodynamic changes, we have used the pulmonary acceleration time as a measure of mean pulmonary artery pressure which we have shown to be reproducible in both normal volunteer [35] and patient studies [36] and has been shown to have a good correlation with catheter-derived measures in both our own hands and those of other workers [22,37], although it is generally accepted that the pulmonary acceleration time cannot be used to precisely measure MPAP in a patient population [22]. We have previously employed these methods to study the pulmonary vascular effects of vasoconstrictors [8] and vasodilators [38], giving results which concur well with invasive studies of the same agents [7,39] although some studies have shown a poor correlation between changes in pulmonary acceleration time and catheter-measured MPAP [40,41]. Doppler echocardiography is also a well-validated and reproducible measure of cardiac output [42]. We do not think that the observed changes in TPR are likely to be confounded by alterations in wedge pressure since in cor pulmonale the increase in resistance is confined to the precapillary vasculature.

In addition to beneficial pulmonary haemodynamic changes, the inclusion of a placebo limb has allowed us to examine the effects of ANG II receptor blockade on plasma aldosterone levels. Plasma aldosterone is known to increase following the assumption of the upright posture and decrease following resumption of the supine posture [43,44], an effect thought to be due to changes in both aldosterone secretion and clearance [45]. As expected with supine rest, plasma aldosterone levels fell on both study days with falls on the placebo day similar to those seen in normal volunteers [43]. In addition, however, treatment with losartan appears to have additional beneficial effects with plasma aldosterone significantly lower 4 h after dosing. This reduction in plasma aldosterone is likely to reflect a reduction in ANG-II-mediated aldosterone biosynthesis and secretion, and although ACE inhibitors have been shown to have a similar effect, ANG II blockers may theoretically produce greater aldosterone suppression since bradykinin may indirectly potentiate aldosterone release [46]. By lowering plasma aldosterone, ANG II receptor blockade may act to prevent excessive salt and water retention, which may be an important precipitating factor in acute exacerbations of cor pulmonale [47]. The RAAS also has significant trophic effects on vascular and cardiac muscle [48], whether lowering aldosterone levels and inhibiting the trophic effects of ANG II is sufficient to inhibit these mitogenic effects is unknown but may be important in arresting the cardiopulmonary remodelling characterising this condition.

So to conclude, in addition to previous work demonstrating that ANG II receptor blockade can attenuate the acute hypoxic pulmonary vasoconstrictor response, we have now shown that the acute administration of losartan has beneficial pulmonary haemodynamic and endocrine effects

without worsening systemic oxygenation. Whether manipulating the renin–angiotensin–aldosterone system with ACE inhibitors or angiotensin II blockade may be of therapeutic value in hypoxaemic cor pulmonale by inhibiting cardiopulmonary remodelling or reducing right ventricular afterload can only be answered by conducting large, long-term follow-up studies.

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Hypoxaemia and release of endothelin-1

Robert I Cargill, David G Kiely, Roland A Clark, Brian J Lipworth

Abstract

Background – Secretion of the vasoconstrictor peptide endothelin-1 from vascular endothelium is increased by various stimuli. Whether hypoxaemia affects plasma levels of endothelin-1 in humans is unknown, but this may be important in the haemodynamic response to hypoxaemia. The plasma endothelin-1 concentrations in hypoxaemic humans has therefore been measured.

Methods – Plasma levels of endothelin-1 were measured by specific radioimmunoassay in 10 control subjects at rest and following 30 minutes of acute hypoxaemia (SaO_2 75–80%) induced by breathing a nitrogen/oxygen mixture, and in 10 patients with hypoxaemic cor pulmonale.

Results – The plasma endothelin-1 concentration in control subjects was increased from a mean (SE) of 0.90 (0.11) pmol/l at baseline to 2.34 (0.34) pmol/l during hypoxaemia. In patients with cor pulmonale the plasma endothelin-1 concentration was 2.96 (0.34) pmol/l, raised in comparison with control subjects at rest but similar to levels in controls during hypoxaemia.

Conclusions – Plasma levels of endothelin-1 were increased by hypoxaemia in humans. The raised levels observed in patients with cor pulmonale may largely be attributable to the effects of hypoxaemia, although the pathophysiological significance of these observations remains to be established.

(Thorax 1995;50:1308–1310)

Keywords: endothelin, cor pulmonale, hypoxaemia.

The endothelins are a group of structurally similar peptides synthesised by vascular endothelium. These peptides act via two specific receptors. Stimulation of the type A endothelin receptor (ET_A), the main subtype found on vascular smooth muscle cells,¹ results in profound and longlasting vasoconstriction, whilst stimulation of the type B receptor (ET_B) causes release of vasodilator metabolites and transient vasodilation.² Interestingly, however, in rat pulmonary resistance vessels in vitro ET_B activation may be important in mediating endothelin-1 induced vasoconstriction.³ In humans, endothelin-1 is the most potent vaso-

constrictor substance known through its relatively selective activation of the ET_A receptor.⁴ A role in the maintenance of vascular tone is suspected but this remains speculative at present.

Synthesis of this peptide is increased by a number of humoral and physical stimuli, including hypoxia, which may be pathophysiologically relevant.⁵ In vitro studies have shown that acute hypoxia increases endothelin-1 production by human endothelial cells⁶ whilst, in experimental animals in vivo, hypoxia increases plasma concentrations of endothelin-1.⁷ The effect of hypoxaemia on endothelin-1 in humans is unknown but may be relevant in the cardiovascular adaptations to hypoxaemia and the circulatory abnormalities seen in conditions such as cor pulmonale.

We have therefore studied the effects of hypoxaemia on plasma endothelin-1 levels in healthy subjects and also in patients with hypoxaemic cor pulmonale.

Methods

SUBJECTS

Normal controls

Ten young male volunteers of mean (SE) 28.1 (2.2) years were studied. None was taking prescribed medication and all had a normal clinical history and examination, 12-lead electrocardiogram, echocardiogram, and haematological and biochemical screen, and forced expiratory volume in one second (FEV_1) of >90% predicted.

Cor pulmonale patients

Ten patients (six men) of mean (SE) age 73.4 (1.7) years with clinically stable cor pulmonale secondary to chronic obstructive pulmonary disease were studied. All had obstructive pattern spirometry (FEV_1 /forced vital capacity (FVC) <70%), arterial hypoxaemia while breathing air (PaO_2 <8.0 kPa), and had or gave a history of having peripheral oedema despite normal left ventricular function (on echocardiogram or radionuclide ventriculography), normal renal function (serum creatinine <120 mmol/l), and normal serum albumin (>35 g/l). Patients with other significant cardiovascular disease were excluded.

In this group, FEV_1 in litres was 0.75 (0.06) (range 0.36–1.11), FEV_1 as % of predicted 33.8 (5.2) (range 19–61), PaO_2 on air 6.30

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(0.34) kPa (range 4.35–7.80), and P_{aCO_2} on air 6.17 (0.50) kPa (range 3.84–8.80).

PROTOCOL

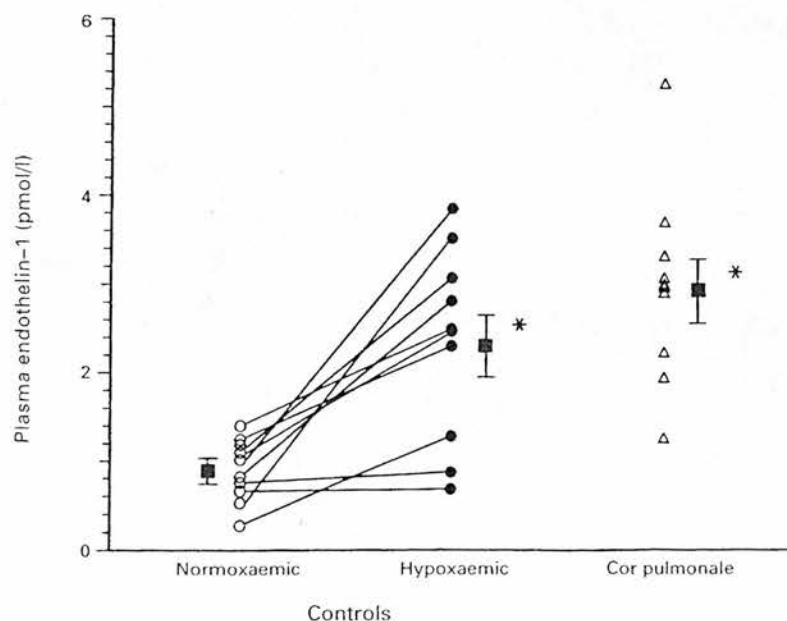
Informed consent was obtained to the protocol previously approved by the Tayside committee for medical research ethics. Baseline venous blood samples were taken into chilled EDTA tubes after 30 minutes supine rest while breathing air. Control subjects only were then rendered hypoxaemic (steady state SaO_2 75–80% measured by pulse oximetry) for 30 minutes by breathing a variable nitrogen/oxygen mixture before taking a further blood sample.

ENDOTHELIN-1 ASSAY

Samples were centrifuged at 2000 rpm at 4°C for 15 minutes and stored at -70°C. Assay was carried out using a commercially available radioimmunoassay kit (Nicholls Institute Diagnostics, San Juan Capistrano, CA, USA) after solid phase extraction on C18 silica columns. The lower limit of detection was 0.4 pmol/l and the intra-assay coefficient of variation was 4.5%. The anti-endothelin antibody used was 100% specific for endothelin-1 and had cross-reactivity of 96% with endothelin-3 and 7% with proendothelin.

DATA ANALYSIS

After testing for normality of distribution, between group comparisons were made by analysis of variance followed by Duncan's multiple range testing with $p < 0.05$ considered significant.⁸ Results are expressed as mean (SE) with 95% confidence intervals (CI) for significant differences.



Levels of endothelin-1 in 10 controls when normoxaemic (○) and following 30 minutes hypoxaemia (●), and in 10 patients with cor pulmonale (△). Boxes indicate sample means with standard error bars and asterisks indicate values significantly ($p < 0.05$) different from normoxaemic controls.

Results

The plasma endothelin-1 concentration in normoxaemic controls was 0.90 (0.11) pmol/l. After 30 minutes hypoxaemia it increased significantly to 2.34 (0.34) pmol/l (95% CI for mean difference 0.41 to 2.48). In patients with cor pulmonale the plasma endothelin-1 concentration was 2.96 (0.34) pmol/l, significantly greater than control subjects when normoxaemic (95% CI for mean difference 1.02 to 3.09) but not when hypoxaemic.

The plasma endothelin-1 concentration therefore increased 2.6-fold in response to hypoxaemia in controls and was raised 3.3-fold in patients with cor pulmonale compared with normoxaemic controls. Results from individual subjects with sample means are depicted in the figure.

Discussion

These findings indicate that hypoxaemia increases plasma levels of endothelin-1 in humans. In patients with cor pulmonale, endothelin-1 was increased to levels comparable to those in normal subjects rendered acutely hypoxaemic. It is interesting to note that baseline levels in controls were similar to those observed in other series,⁹ and that the increase following hypoxaemia and in patients with cor pulmonale is comparable to the rise seen in normal subjects at high altitude.¹⁰

These observations in humans are largely confirmatory of *in vitro* and animal studies which have shown hypoxia to be a potent stimulus for endothelin-1 synthesis and gene expression.^{6,7} In normal humans Therkelson *et al* found that 15 minutes of hypoxaemia caused only a small increase in plasma endothelin-1 which was not statistically significant, perhaps due to the shorter duration of the stimulus.¹¹ Abnormally high levels of endothelin-1 have been described in a series of patients with pulmonary hypertension of varying aetiology, not all of whom were hypoxaemic,⁹ and thus other stimuli may also be implicated. In the present series, by excluding subjects with other significant cardiovascular diseases such as hypertension and congestive heart failure, we feel hypoxaemia is the most significant stimulus responsible for increased endothelin-1 levels in cor pulmonale. The *in vitro* evidence would suggest that the increased endothelin-1 levels observed were due to increased synthesis although, as endothelin-1 clearance mechanisms in humans have not been fully characterised,⁵ the possibility of decreased removal cannot be discounted.

The potent vasoactive properties of endothelin-1 may also be responsible for some of the circulatory abnormalities seen during hypoxaemia, although plasma levels may not accurately reflect local concentrations and hence vasoconstrictor activity. Whether endothelin-1 acts as a mediator of acute hypoxic pulmonary vasoconstriction is unknown. Support for this hypothesis might be drawn from studies using endothelin receptor blockers which can attenuate acute hypoxic pulmonary vasoconstriction in rats¹² and prevent development

of pulmonary hypertension following chronic hypoxia.¹³ These drugs now need to be tested in humans where it is likely that endothelin-1 plays a significant part in the cardiovascular response to hypoxaemia.

Manipulation of the endothelin system may therefore be a useful measure in patients with hypoxaemic lung disease, either to prevent or treat the cardiopulmonary consequences of chronic hypoxaemia.

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Cardiopulmonary effects of endothelin-1 in man

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Cardiopulmonary effects of endothelin-1 in man

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Abstract

Objectives: Endothelin-1 levels are elevated in a number of conditions characterised by impaired cardiovascular performance and abnormal vasoconstriction such as congestive cardiac failure and primary and secondary pulmonary hypertension. The aim of the present study was to assess the effects of the vasoconstrictor peptide endothelin-1 on pulmonary and systemic haemodynamics and cardiovascular performance in normal man. **Methods:** Ten healthy male volunteers were studied on two occasions in a randomised, double-blind, placebo-controlled, cross-over study and received systemic infusions of either endothelin-1 (0.75, 1.5 and 3 pmol · kg⁻¹ · min⁻¹ for 30 min each) or saline placebo. Systemic and pulmonary haemodynamic parameters were monitored non-invasively by pulsed-wave Doppler, as were parameters of left and right ventricular diastolic filling and inotropic state. Effects on renin–angiotensin and natriuretic peptide system activity were also measured. **Results:** Endothelin-1 infusion produced dose-related falls in heart rate, stroke volume and cardiac output. Systemic vascular resistance (SVR) increased from 1156 ± 57 to 1738 ± 115 dyn · s · cm⁻⁵, and total pulmonary vascular resistance (TPR) increased from 142 ± 12 to 329 ± 22 dyn · s · cm⁻⁵. Endothelin-1 caused significant impairment of left and right ventricular diastolic filling, even at a low dose which had no pulmonary or systemic pressor effects. Electromechanical and Doppler acceleration indices of inotropic state were also significantly impaired. Activity of the renin–angiotensin system was suppressed by endothelin-1 whilst plasma levels of atrial natriuretic peptide (ANP) were unchanged. **Conclusions:** Thus, in addition to systemic and pulmonary pressor effects our results suggest that endothelin-1 impairs overall cardiovascular performance by causing diastolic dysfunction and acting as a negatively inotropic agent. These effects were associated with compensatory changes in the renin–angiotensin system.

Keywords: Endothelin; Vasoconstriction; Endothelium; Renin–angiotensin–aldosterone system; ANP; Human

1. Introduction

Endothelial function is an important regulator of vascular tone although the physiological roles of the endothelium-derived relaxing and constricting factors and their functional antagonism remain the subject of much ongoing research effort. In terms of vasoconstriction, the family of peptides known as the endothelins have emerged as being of major importance. These are a group of structurally similar peptides synthesised by vascular endothelium which act via two specific receptors. Stimulation of the type A endothelin receptor (ET_A) results in a gradual onset but long-lasting vasoconstriction whilst stimulation of the type B receptor (ET_B), causes release of vasodilator metabolites

and transient vasodilatation but may also be involved in the vasoconstrictor response [1]. ET_A receptors are the main subtype found on vascular smooth muscle cells [2] and have also been localised to the heart and adrenal gland [3]. In vitro, endothelin-1 is the most potent vasoconstrictor substance known through its relatively selective activation of the G-protein-linked ET_A receptor [4] as well as being the most abundant endothelin isoform in human plasma [5].

Release of endothelin-1 from endothelial cells is stimulated by a variety of physical and chemical stimuli in vitro [1] whilst in humans in vivo, hypoxia and angiotensin II increase plasma concentrations of endothelin-1 [6,7]. Abnormalities of circulating endothelin-1 concentrations have also been observed in a number of conditions characterised

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by abnormal vasoconstriction such as hypertension [8], congestive heart failure (CHF) [9], primary pulmonary hypertension (PPH) [10], and hypoxaemic cor pulmonale [6], raising the possibility that endothelin-1 may play a pathogenic role in both cardiovascular and cardiopulmonary disease. As the physiological effects of endothelin-1 in man have not been fully studied, a precise understanding of this role remains elusive.

Although in previous *in vivo* studies in man, endothelin-1 increased systemic blood pressure [11] and caused renal vasoconstriction [12], the effects of endothelin-1 on the pulmonary vasculature and cardiovascular performance are not known. Furthermore, the design of some studies [11] has not allowed for the prolonged action of endothelin-1 and, as such, may have produced misleading information.

We have therefore evaluated the effects of systemic endothelin-1 administration in healthy volunteers. This study documents effects on systemic and pulmonary haemodynamics, diastolic ventricular function, inotropicity and neurohormonal activation.

2. Methods

2.1. Subjects

Ten healthy, non-obese, male volunteers, age (mean \pm s.e.m.) 26.1 ± 1.4 years were studied. All had normal clinical history and examination, 12-lead electrocardiogram, echocardiogram and haematological and biochemical screen. Subjects refrained from alcohol, tobacco and caffeine for at least 12 h and had taken no medications for at least 1 month before the study. Informed consent was obtained to the study protocol previously approved by the Tayside Committee for Medical Research Ethics and this investigation conforms with the principles outlined in the Declaration of Helsinki.

2.2. Protocol

Subjects were studied at the same time of day on 2 occasions at least 1 week apart in a randomised, double-blind, cross-over design. Subjects were randomised prior to commencement of the study with both subject and echocardiographer (DGK) blinded with respect to the infusate. After completion of the final study day and of all measurements and calculations the active and placebo days were revealed to allow statistical analysis to be performed. On arrival at the clinical laboratory, intravenous cannulae were sited in both forearms for infusion (left) and blood sampling (right). Subjects then remained supine, turned slightly on the left side throughout the study. After allowing at least 30 min rest to achieve baseline haemodynamic parameters, infusion of either pharmaceutical grade human endothelin-1 (Clinalfa AG; Laufelfingen, Switzerland) or

identical volume placebo (0.9% saline) was commenced in random order. Endothelin-1 was infused at $0.75 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 30 min, then increased to $1.5 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 30 min and then to $3.0 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for a further 30 min. Infusions were then discontinued and subjects monitored for a further 60 min. Measurements were made at baseline, at the end of each infusion dose period and 30 and 60 min after stopping the infusion.

2.3. Measurements

For each Doppler-derived parameter, the mean of 3 consistent waveforms recorded at each time point was used for the purpose of analysis.

2.3.1. Haemodynamic indices

Systolic (SBP), mean (MAP), diastolic arterial blood pressure (DBP) and heart rate were measured using a semi-automatic sphygmomanometer (Vital Signs Monitor; Critikon, Tampa, FL, USA) and averaged over 3 sequential readings. To measure cardiac output (CO), aortic cross-sectional area (CSA) was first measured by M-mode echocardiography (Vingmed SD50; Vingmed Sound, Horten, Norway) from the left parasternal view at the level of the aortic root. The aortic systolic flow velocity integral (SVI) was then measured by computer analysis of the pulsed-wave Doppler profile of aortic blood flow from the suprasternal notch. Stroke volume ($\text{SV} = \text{SVI} \times \text{CSA}$) and hence CO, as the product of SV and HR, were then calculated on-line. Systemic vascular resistance (SVR) was then calculated as $\text{SVR} = \text{MAP}/\text{CO} \times 80 \text{ dyn} \cdot \text{s} \cdot \text{cm}^{-5}$.

Measurement of pulmonary flow acceleration time (PAT) by pulsed-wave Doppler echocardiography (Vingmed SD50) from the left 3rd/4th intercostal space allowed calculation of mean pulmonary artery pressure (MPAP) using the regression equation $\text{MPAP} = 73 - (0.42 \times \text{PAT})$ which has been shown to have a good correlation with MPAP measured simultaneously during cardiac catheterisation in patients with pulmonary hypertension [13]. Total pulmonary vascular resistance (TPR) was then calculated as $\text{TPR} = \text{MPAP}/\text{CO} \times 80 \text{ dyn} \cdot \text{s} \cdot \text{cm}^{-5}$. The coefficients of variability (CV) for the Doppler measurements in this study were: SV 4.9%, CO 9.3%, MPAP 4.5%.

2.3.2. Lusitropic indices

From the apical window, pulsed-wave Doppler analysis of mitral and tricuspid diastolic flow (Vingmed SD50) was combined with simultaneous phonocardiography with the microphone (Siemens AG; Munich, Germany) positioned over the 2nd left intercostal space. Recordings were all made during expiration with a display sweep speed of 100 mm/s. Transmitral and transtricuspid flow was analysed after adjusting sample volume depth to yield maximal E-wave velocities with clearly defined flow velocity en-

velopes. Aortic and pulmonary components of the second heart sound were identified on the phonocardiogram trace by noting closure artefacts from superimposition of aortic and pulmonary Doppler flow profiles.

From diastolic transmitral and transtricuspid flow, maximal velocities of the early ($E_{v_{max}}$) and atrial ($A_{v_{max}}$) components of flow were measured, and the E/A ratio was calculated. The isovolumic relaxation time (IVRT) was calculated for the left ventricle as the time in milliseconds from the aortic component of the phonocardiogram second heart sound to the onset of diastolic transmitral flow, and for the right ventricle, the time from the pulmonary component of the phonocardiogram second heart sound to the onset of diastolic transtricuspid flow. The CV for these measurements were: mitral $E_{v_{max}}$ 12.2%, mitral $A_{v_{max}}$ 17.2%, transmitral E/A ratio 12.4%, left ventricular IVRT 13.8%, tricuspid $E_{v_{max}}$ 8.8%, tricuspid $A_{v_{max}}$ 14.2%, transtricuspid E/A ratio 14.8%, right ventricular IVRT 29.1%.

2.3.3. Inotropic indices

Simultaneous lead II ECG, phonocardiogram (Siemens AG) and pulsed-wave Doppler ascending aortic blood flow (Vingmed SD50) were recorded as described. From these recordings, the following variables were measured: aortic peak acceleration (Acc_{peak}), aortic mean acceleration (Acc_{mean}) and electromechanical systole (QS_2) from ECG Q-wave to the second heart sound on phonocardiogram.

QS_2 was corrected for changes in HR according to standard criteria [14] and is denoted QS_2I . The CV values for these measurements were: Acc_{peak} 18.7%, Acc_{mean} 12.5% and QS_2I 3.5%.

2.3.4. Laboratory analyses

Following collection, all samples were kept on ice until centrifugation at 3000 rpm for 15 min at 4°C. Plasma sodium and potassium were measured using an internal caesium standard flame photometer (Instrumentation Laboratory, Milan, Italy). Samples for plasma aldosterone assay were collected into chilled lithium–heparin tubes and for measurement of plasma renin activity (PRA) into chilled EDTA tubes and stored at –20°C until assayed in one batch at the end of the study.

Assays were carried out using commercially available radioimmunoassay kits which assayed aldosterone directly (Sorin Biomedica, Saluggia, Italy) and PRA (Sorin Biomedica) by measurement of amount of angiotensin I generated per hour. For assay of atrial natriuretic peptide (ANP), venous blood was collected into EDTA tubes containing 4000 KIU aprotinin before centrifugation and plasma kept at –70°C until assayed in one batch at the end of the study. After solid-phase extraction from plasma, assay was performed using a commercially available radioimmunoassay kit (Incstar Corporation, Wokingham, Berkshire, UK). The intra-assay CV for these assays were: PRA 7.6%, aldosterone 8.3%, ANP 8.0%.

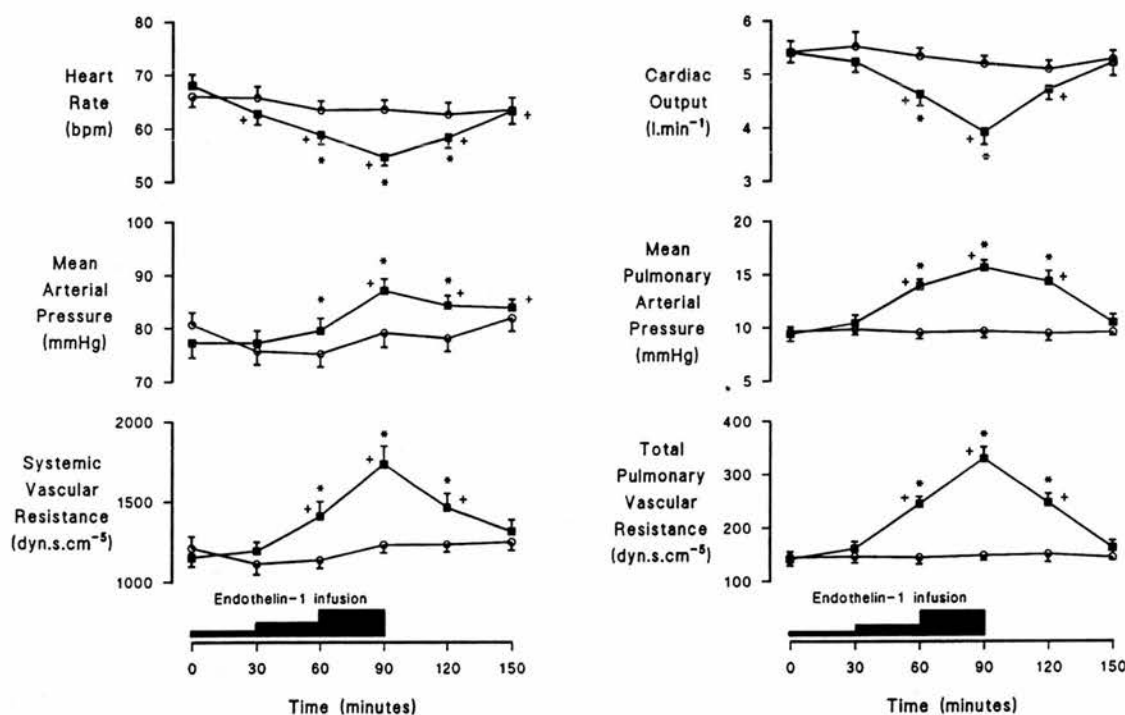


Fig. 1. Systemic and pulmonary haemodynamic changes in response to endothelin-1 (\square) and placebo (\circ) infusions. The doses of endothelin-1 infused are shown in diagrammatic form on the x-axis. Endothelin-1 was infused at $0.75 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 30 min, then increased to $1.5 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 30 min and then to $3.0 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for a further 30 min. * Significant difference from placebo at the same time point. † Significant difference from baseline during endothelin-1 infusion.

2.4. Data analysis

Comparisons were made between active and placebo treatments for each treatment by repeated measures analysis of variance (ANOVA). Where the overall ANOVA was significant, Duncan's multiple range testing was used to determine differences at individual time points. A *P*-value of less than 0.05 was considered significant and results are expressed as means \pm s.e.m.

3. Results

There were no significant adverse effects during the study although one subject felt transiently nauseous and sweaty during maximal rate infusion of endothelin-1.

3.1. Haemodynamic effects

Baseline conditions on each study day were similar for all haemodynamic parameters measured. Endothelin-1 had significant systemic pressor effects compared with placebo, where MAP was elevated following medium- and high-dose endothelin-1 infusion and remained significantly greater than placebo for 30 min after stopping the infusion (Fig. 1). Data for SBP and DBP are given in Table 1 where the pattern of changes was similar. HR decreased during medium and high dose endothelin-1 infusion compared with placebo and remained significantly lower than placebo for 30 min after stopping infusions (Fig. 1) whilst SV was only significantly reduced during high dose endothelin-1 infusion (Table 1). CO also decreased during medium and high dose endothelin infusion but was not significantly different from placebo during the recovery period (Fig. 1). Endothelin-1 significantly increased SVR during medium and high dose infusion and remained greater than placebo after 30 min recovery (Fig. 1). In the pulmonary circulation, both MPAP and TPR were increased by medium and high dose infusion, remaining higher than placebo after 30 min recovery (Fig. 1).

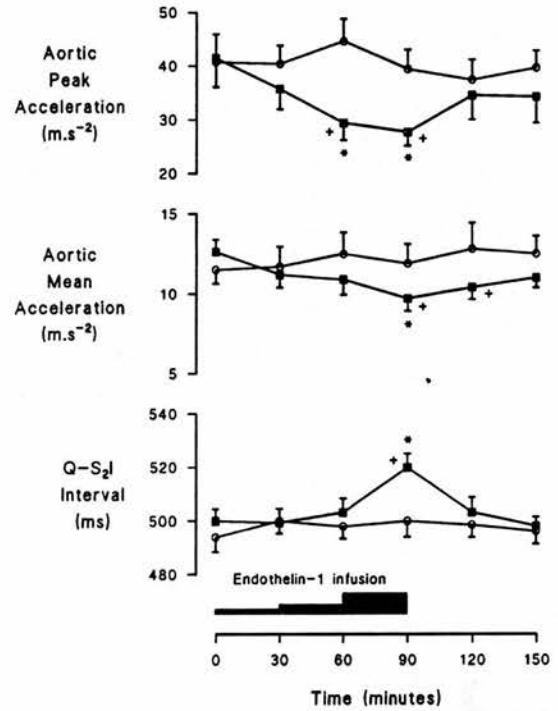


Fig. 2. Changes in inotropic indices in response to endothelin-1 (\square) and placebo (\circ) infusions. The doses of endothelin-1 infused are shown in diagrammatic form on the x-axis. Endothelin-1 was infused at $0.75 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 30 min, then increased to $1.5 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 30 min and then to $3.0 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for a further 30 min. * Significant difference from placebo at the same time point. † Significant difference from baseline during endothelin-1 infusion.

3.2. Inotropic effects

Aortic Acc_{mean} and Acc_{peak} were similar at baseline on each study day. Both parameters decreased during endothelin-1 infusion to levels significantly lower than placebo during medium and high dose infusion for Acc_{peak} (Fig. 2) and at high dose only for Acc_{mean} (Fig. 2). Compared with placebo, QS_2I was significantly prolonged only during high dose endothelin-1 infusion (Fig. 2).

Table 1
Systemic haemodynamics in response to endothelin-1 and placebo infusions

		Time (min)					
		0	30	60	90	120	150
SBP (mmHg)	Placebo	112 ± 2.9	107 ± 2.9	106 ± 2.5	108 ± 3.5	110 ± 2.8	110 ± 3.6
	Active	108 ± 2.5	107 ± 2.2	109 ± 2.1	$114 \pm 3.5^{* \dagger}$	113 ± 1.9	112 ± 1.9
DBP (mmHg)	Placebo	61 ± 2.5	58 ± 2.5	57 ± 2.5	60 ± 2.2	60 ± 1.7	61 ± 2.2
	Active	57 ± 2.0	57 ± 1.8	$62 \pm 2.1^{* \dagger}$	$67 \pm 3.2^{* \dagger}$	$64 \pm 1.8^{* \dagger}$	$64 \pm 2.4^{\dagger}$
SV (ml)	Placebo	90 ± 4.0	89 ± 4.2	88 ± 3.6	86 ± 3.2	87 ± 4.7	89 ± 4.4
	Active	90 ± 3.8	88 ± 4.4	85 ± 4.6	$81 \pm 5.0^{\dagger}$	90 ± 4.6	90 ± 4.3

Absolute values (mean \pm s.e.m.) of systolic and diastolic blood pressure (SBP, DBP) and stroke volume (SV) during placebo and endothelin-1 infusion.

* Significant difference from placebo at the same time point.

† Significant difference from baseline during endothelin-1 infusion.

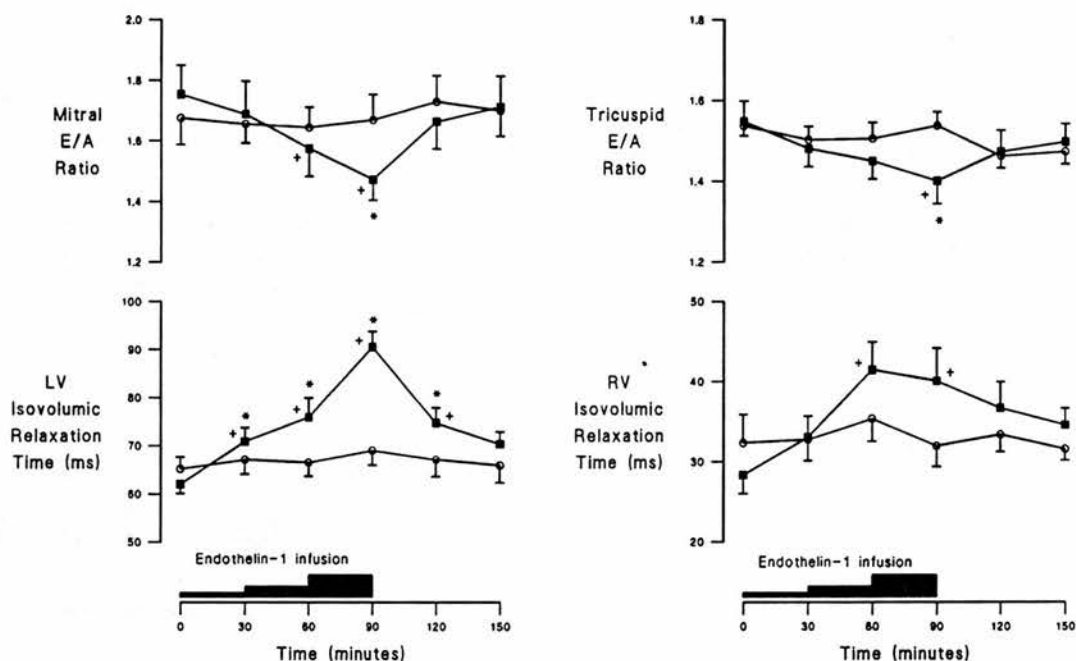


Fig. 3. Echo-Doppler parameters of left (LV) and right (RV) ventricular filling in response to endothelin-1 (□) and placebo (○) infusions. The doses of endothelin-1 infused are shown in diagrammatic form on the x-axis. Endothelin-1 was infused at $0.75 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 30 min, then increased to $1.5 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 30 min and then to $3.0 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for a further 30 min. * Significant difference from placebo at the same time point. † Significant difference from baseline during endothelin-1 infusion.

Table 2
Left and right ventricular filling parameters in response to endothelin-1 and placebo infusions

All units ($\text{m} \cdot \text{s}^{-1}$)		Time (min)					
		0	30	60	90	120	150
Mitral	Placebo	70 ± 4.1	69 ± 4.3	71 ± 4.0	68 ± 3.4	70 ± 3.2	68 ± 4.0
$E V_{\max}$	Active	71 ± 3.9	71 ± 4.2	62 ± 3.3 *†	59 ± 3.4 *†	63 ± 3.0 †	71 ± 4.4
Mitral	Placebo	42 ± 1.9	42 ± 1.7	43 ± 1.6	41 ± 1.7	41 ± 1.6	41 ± 1.8
$A V_{\max}$	Active	41 ± 1.7	43 ± 2.0	40 ± 1.8	40 ± 2.4	38 ± 1.8	42 ± 2.7
Tricuspid	Placebo	47 ± 1.7	45 ± 1.2	47 ± 1.3	46 ± 1.3	46 ± 1.4	46 ± 1.2
$E V_{\max}$	Active	46 ± 2.1	46 ± 2.1	45 ± 1.8	43 ± 1.7	42 ± 0.7 *†	43 ± 1.0 †
Tricuspid	Placebo	31 ± 1.0	30 ± 1.1	31 ± 1.2	30 ± 0.9	32 ± 1.1	31 ± 0.8
$A V_{\max}$	Active	30 ± 0.9	32 ± 1.4	31 ± 1.0	31 ± 1.5	29 ± 0.9	29 ± 1.3

Absolute values (mean \pm s.e.m.) of transmitral and transtricuspid E- and A-wave maximal velocity during placebo and endothelin-1 infusion.

* Significant difference from placebo at the same time point.

† Significant difference from baseline during endothelin-1 infusion.

Table 3
Serum electrolytes and atrial natriuretic peptide in response to endothelin-1 and placebo infusions

		Time (min)					
		0	30	60	90	120	150
Na ($\text{mmol} \cdot \text{l}^{-1}$)	Placebo	138 ± 0.7	138 ± 0.6	138 ± 0.8	138 ± 0.6	136 ± 0.8	138 ± 1.0
	Active	137 ± 0.7	138 ± 0.7	137 ± 0.9	137 ± 0.8	137 ± 0.6	138 ± 0.6
K ($\text{mmol} \cdot \text{l}^{-1}$)	Placebo	4.0 ± 0.08	4.1 ± 0.04	4.1 ± 0.06	4.1 ± 0.05	4.0 ± 0.06	4.1 ± 0.08
	Active	4.1 ± 0.07	4.2 ± 0.11	4.1 ± 0.08	4.2 ± 0.06	4.2 ± 0.08	4.2 ± 0.09
ANP ($\text{pmol} \cdot \text{l}^{-1}$)	Placebo	4.82 ± 1.53	4.61 ± 0.59	4.36 ± 0.43	4.05 ± 0.49		
	Active	5.57 ± 0.68	5.97 ± 0.43	5.71 ± 0.79	6.00 ± 0.71		

Absolute values (mean \pm s.e.m.) of serum sodium (Na), potassium (K) and atrial natriuretic peptide (ANP) during placebo and endothelin-1 infusion. There were no significant differences between endothelin-1 and placebo treatments or with time.

3.3. Lusitropic effects

All lusitropic parameters were similar at baseline on each study day. Mitral and tricuspid E/A ratio decreased during endothelin-1 infusion and were significantly different from placebo during high dose infusion only (Fig. 3). These changes were due to falls in $E_{v_{max}}$ rather than increases in $A_{v_{max}}$ (Table 2). IVRT was a more sensitive marker of impaired ventricular filling where in the left ventricle IVRT was prolonged by endothelin-1 even at the lowest infusion rate compared with placebo (Fig. 3) whilst in the right ventricle, IVRT during endothelin-1 infusion was not significantly different from placebo but was prolonged from baseline during medium and high dose infusion (Fig. 3).

3.4. Endocrine effects

Plasma sodium and potassium were similar at baseline on both study days and did not change significantly during either placebo or endothelin-1 infusion (Table 3). PRA was similar at baseline on both days and fell significantly from baseline after 90 min of placebo infusion (Fig. 4). The fall during endothelin-1 infusion was however more rapid and levels were significantly lower than baseline after low dose infusion and continued to fall up to 60 min after stopping infusion. Levels were significantly lower than placebo

during medium and high dose endothelin-1 infusion and throughout the recovery period (Fig. 4). There was no difference in plasma aldosterone either at baseline or following endothelin-1 infusion compared with placebo. Levels fell significantly from baseline after 60 min of placebo infusion and then began to plateau (Fig. 4). Levels also fell following endothelin-1 infusion, reaching a significant nadir at the medium dose (Fig. 4). No effect on plasma ANP levels was observed during either placebo or endothelin-1 infusion (Table 3).

4. Discussion

Although endothelin-1 was initially characterised as a vasoconstrictor, it is clear from the present study that in addition to pressor effects, endothelin-1 may also have direct effects on myocardial function in man. In comparison with animal studies [15], these effects were observed using relatively low doses of endothelin-1, although just exceeding the previously highest reported dose in humans [11]. We have administered endothelin-1 exogenously in a range of doses, with the lowest dose achieving levels of endothelin-1 found in pathophysiological states whilst the higher doses will have achieved so-called 'pharmacological' concentrations. Unlike previous studies we have not measured plasma endothelin-1 levels as this appears highly variable between individuals during infusions of endothelin-1 [11], and as endothelin-1 has paracrine rather than endocrine actions, plasma levels may not be comparable with levels measured in pathological states. In these conditions plasma levels are likely to represent 'spillover' with much higher levels of endothelin-1 found abuminally. It is for these reasons that our dose range included infusions that were known to achieve pathophysiological concentrations of endothelin-1 and greater. The relatively longer infusion times in the present investigation have also allowed a clearer definition of the dose-related effects of endothelin-1 than would be obtained with shorter dose increments.

4.1. Study limitations

The parameters in this study were measured non-invasively due to ethical considerations and as such have associated limitations. Although our indices of inotropy and lusitropy have previously been validated [16–18], these measures can be affected to greater or lesser degrees by loading conditions and we have alluded to this in the text. Doppler echocardiography was used to measure haemodynamic changes in the pulmonary circulation. Pulmonary acceleration time, which we have shown to be reproducible in our own hands, was used to estimate mean pulmonary artery pressure which has been shown to have a good correlation with catheter-derived measures, although in a patient population it cannot be accurately used to

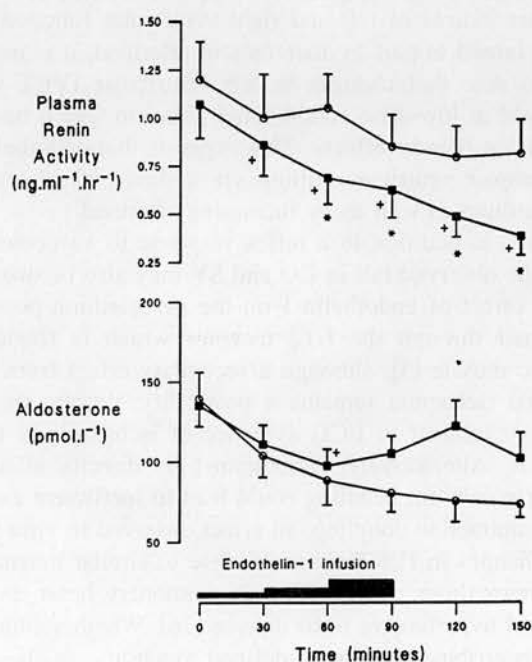


Fig. 4. Changes in renin-angiotensin system activity in response to endothelin-1 (□) and placebo (○) infusions. The doses of endothelin-1 infused are shown in diagrammatic form on the x-axis. Endothelin-1 was infused at $0.75 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 30 min, then increased to $1.5 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for 30 min and then to $3.0 \text{ pmol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ for a further 30 min. * Significant difference from placebo at the same time point. + Significant difference from baseline during active infusion.

precisely estimate pulmonary artery pressure [13,19]. Although estimation of changes in pulmonary artery pressure over time may be accurate, some caution should be used when taking these values for further calculations. We looked at two measures of pulmonary vasoconstriction changes in MPAP and TPR. A limitation of this methodology is that TPR neglects the downstream pressure of the pulmonary circulation, namely left atrial pressure, as conventionally assessed by pulmonary capillary wedge pressure (PCWP). Although this may be increased if left ventricular function is impaired, as was the case in this study, the magnitude of the changes observed in the pulmonary vascular bed suggest an effect on the pre-capillary vasculature. With respect to Doppler measures of cardiac output, these are accepted as a relatively accurate measure which can be made in most patients [20]; the major source of error is generally accepted as measurement of the annular diameter and this would be mitigated by the cross-over design of the study. In addition, it remains difficult to separate primary from secondary effects of endothelin-1 on cardiopulmonary performance using this protocol, although some of the data do support direct effects on the heart.

4.2. Systemic and pulmonary haemodynamic effects

The haemodynamic effects of endothelin-1 in man are not well characterised although our haemodynamic data agree with findings from animal studies [15]. Our study confirms that endothelin-1 is a potent *in vivo* systemic vasoconstrictor and it is interesting to note that MAP and SVR were elevated compared to baseline for at least 30 min after stopping the infusion, indicating prolonged vasoconstriction. This is in keeping with *in vitro* data [21] and with the observation that although plasma levels fall rapidly after stopping endothelin-1 infusion, the increase in blood pressure is maintained [11]. The relative bradycardia and fall in CO during endothelin infusion may have been due to a vagal reflex response to systemic vasoconstriction, myocardial ischaemia or due to a direct effect on the conducting system of the heart.

Most previous studies have neglected the action of endothelin-1 on pulmonary haemodynamics. We have tried to characterise these using non-invasive techniques which we have previously employed to study the pulmonary vascular effects of vasoconstrictors [22] and vasodilators [23], giving results which concur well with invasive studies of the same agents [24,25]. We have measured TPR and not pulmonary vascular resistance (PVR) and as such have neglected the downstream pressure of the pulmonary circulation. Our results suggest that endothelin-1 impairs diastolic function and acts as a negatively inotropic agent, and as such have reasons to believe that left atrial pressure did increase in these subjects. Although we cannot conclude that PVR was increased in our subjects, the magnitude of the changes in the pulmonary vascular bed is consistent

with a vasoconstrictor effect for endothelin-1, an effect that would be predicted from *in vitro* and animal studies [15,26]. At a receptor level, although still attributable to endothelin-1, some debate exists about the role that activation of the ET_B receptor plays in producing pulmonary vasoconstriction [27].

4.3. Inotropic and lusitropic effects

Our findings also support the hypothesis that endothelin-1 has negative inotropic effects and impairs ventricular filling in humans and is similar to the findings of previous animal studies [28]. We have used echo-Doppler parameters and systolic time intervals to assess left ventricular contractility as both QS_2I and Acc_{peak} are thought to be relatively independent of loading conditions. In this respect, *in vitro* and *in vivo* work have shown that changes in QS_2I reflect inotropic influences and that load conditions only have a proportionately small effect on this interval [29], although conflicting data exist regarding Doppler-derived parameters of ascending aortic flow [30,31]. Studies examining the effect of afterload on trans-mitral flow patterns have been hampered by difficulties involving sympathetic and neurohormonal activation; however, the general consensus is that afterload decreases early filling velocity and E/A ratio [32]. The IVRT also varies with changes in afterload and prolongation of the IVRT is directly related to the natural logarithm of aortic closing pressure [33]. Although the changes in IVRT and our Doppler indices of left and right ventricular function can be explained in part by increases in afterload, it is important to note that changes in left ventricular IVRT were observed at low-dose endothelin-1 infusion which had no significant pressor effects. This suggests that endothelin-1 may impair ventricular filling via a direct effect on the myocardium as well as by increasing afterload.

Thus, in addition to a reflex response to vasoconstriction, the observed fall in CO and SV may also be due to a direct effect of endothelin-1 on the myocardium possibly mediated through the ET_A receptor which is found on cardiac muscle [3], although a secondary effect from myocardial ischaemia remains a possibility, despite the absence of clinical or ECG evidence of ischaemia in these subjects. Alternatively, endothelin-1 by directly affecting myocyte calcium handling could lead to inefficient excitation-contraction coupling, an effect observed *in vitro* [34]. The changes in IVRT observed were of similar magnitude to abnormalities associated with pulmonary heart disease [35] and hypertensive heart disease [36]. Whether endothelin-1 contributes to the ill-defined syndrome of diastolic heart failure is also unknown.

4.4. Endocrine effects

In terms of effects on the renin-angiotensin system (RAS), endothelin-1 has been variously reported to have

stimulatory and inhibitory actions [1]. In vitro studies would indicate that endothelin-1 inhibits renin release [37] and stimulates aldosterone release in cell culture [38], although in humans in vivo this effect may be offset by renal vasoconstriction [12] increasing renin release and hence aldosterone, as no effect on these parameters has been reported [11,12]. The findings of the present study are therefore of interest where the inclusion of a placebo limb allows the prolonged, time-dependent fall in parameters of RAS activity to be considered. We observed PRA to fall in comparison with placebo after medium dose infusion, an effect which continued throughout the remainder of the study even when haemodynamic parameters had normalised. This therefore provides indirect evidence for a direct effect on renin production rather than a phenomenon secondary to renal vasoconstriction. The pattern with regard to aldosterone was different where, although not significantly different from placebo, there was a biphasic response during endothelin-1 infusion. This apparent dissociation of components of the RAS may be related to increases in ACTH-mediated effects on aldosterone secretion. In addition, despite the increase in cardiac afterload, no change in plasma ANP concentration was observed.

Contrary to a previous study in humans [11], we found no significant effects on plasma electrolytes, a finding in agreement with animal studies [15]. The possibility that mild haemolysis was responsible for this finding has been raised [11] and thus different peptide or infusion preparation practices may have been responsible.

5. Conclusions

We have shown that endothelin-1, in addition to potent pressor effects, has adverse effects on both inotropic and lusitropic parameters. These new findings may be important in considering the pathophysiological role of endothelin-1 in both cardiovascular and cardiopulmonary disease.

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Nitric oxide: an important role in the maintenance of systemic and pulmonary vascular tone in man

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Aims The aim of this study was to examine whether nitric oxide (NO) has an important role in maintaining basal vascular tone in normal man by examining the effects of nitric oxide inhibition using N^G -monomethyl-L-arginine (L-NMMA) on systemic and pulmonary haemodynamics.

Methods Ten normal male volunteers 26 ± 1.6 years were studied on two separate occasions in a double-blind, placebo controlled crossover study. They were randomised to receive either a continuous infusion of L-NMMA ($4 \text{ mg kg}^{-1} \text{ h}^{-1}$) with a front loaded bolus (4 mg kg^{-1}) or volume matched placebo. Pulsed wave Doppler echocardiography was used to measure cardiac output (CO), mean pulmonary artery pressure (MPAP) and hence systemic vascular resistance (SVR) and total pulmonary vascular resistance (TPR). Measurements were made prior to infusion (t_0) and after 4, 8, and 12 min (t_1 , t_2 and t_3).

Results Infusion of L-NMMA significantly increased mean arterial blood pressure (MAP), SVR and TPR and significantly reduced heart rate (HR), stroke volume (SV) and CO compared to placebo. These effects were observed at t_1 and persisted during the entire infusion period.

Conclusions These results are consistent with a role for basal nitric oxide generation in the maintenance of basal systemic and pulmonary vascular tone in normal man.

Keywords: nitric oxide, pulmonary circulation, systemic circulation

Introduction

Endothelial function is known to be an important determinant of vascular tone although the physiological roles of the endothelium-derived relaxing and constricting factors and their functional antagonism remain the subject of much ongoing research effort. Much interest surrounds a potent short acting vasodilator, nitric oxide, which is generated by the vascular endothelium through the action of nitric oxide synthase [1]. Studies in animals using systemic infusions of nitric oxide synthase inhibitors such as N^G -monomethyl-L-arginine (L-NMMA) have demonstrated an increase in mean arterial pressure and systemic vascular resistance [2, 3]. Evidence in humans suggesting an important role for nitric oxide generation in the maintenance of systemic vascular tone comes from studies demonstrating that infusion of L-NMMA into the brachial artery results in vasoconstriction [4] and systemic infusions of L-NMMA increase systemic vascular resistance [5].

There is however, some debate as to the role of nitric oxide generation in the maintenance of the low pressure pulmonary circulation where conflicting results exist suggesting the possibility of species specificity [6–10]. Interestingly, studies in animals and humans have suggested that impaired endothelial relaxing activity may contribute to the pathogenesis of pulmonary hypertension [11, 12]. To date only Stamler and his colleagues have examined the effects of

nitric oxide inhibition on pulmonary vascular tone in normal man in a dose ranging study [6]. This study examines for the first time in a placebo controlled manner the effects of nitric oxide inhibition on pulmonary vascular tone in normal man using non-invasive pulsed wave Doppler echocardiography.

Methods

Subjects

Ten healthy, non-obese, male volunteers, age (mean \pm s.e.mean) 26 ± 1.6 years were studied. All had normal clinical history and examination, 12-lead electrocardiogram, echocardiogram and haematological and biochemical screen. Subjects refrained from alcohol, tobacco and caffeine for at least 12 h and had taken no medications for at least 1 month before the study. Informed consent was obtained to the study protocol previously approved by the Tayside Committee for Medical Research Ethics and this investigation conforms with the principles outlined in the Declaration of Helsinki.

Protocol

Subjects were studied at the same time of day on two occasions in a randomized, double blind, cross-over design. Subjects then remained supine, turned slightly on the left side throughout the study. After allowing at least 30 min rest to achieve baseline haemodynamic parameters they

received either a continuous infusion of L-NMMA ($4 \text{ mg kg}^{-1} \text{ h}^{-1}$) with a front loaded bolus (4 mg kg^{-1}) given over 2 min or volume matched placebo (0.9% normal saline). Measurements were made prior to infusion (t_0) and after 4, 8, and 12 min (t_1 , t_2 and t_3).

Measurements

For each Doppler derived parameter, the mean of three consistent waveforms recorded at each time point was used for the purpose of analysis. MAP and HR were measured by semi-automatic sphygmomanometer (Vital Signs Monitor; Critikon, Tampa, FL, USA) and averaged over three sequential readings. To measure CO, aortic cross-sectional area (CSA) was first measured by M-mode echocardiography (Vingmed SD50; Vingmed Sound, Horten, Norway) from the left parasternal view at the level of the aortic root. The aortic systolic flow velocity integral (SVI) was then measured by computer analysis of the pulsed-wave Doppler profile of aortic blood flow from the suprasternal notch. Stroke volume ($\text{SV} = \text{SVI} \times \text{CSA}$) and hence CO, as the product of SV and HR, were then calculated on-line. Measurement of pulmonary flow acceleration time (PAT) by pulsed-wave Doppler echocardiography (Vingmed SD50) from the left 3rd/4th intercostal space allowed calculation of mean pulmonary artery pressure (MPAP) using the regression equation $\text{MPAP} = 73 - (0.42 \times \text{PAT})$ which has been shown to have a good correlation with MPAP measured simultaneously during cardiac catheterisation in patients with pulmonary hypertension in both our own hands [13] and those of other workers [14]. The coefficients of variability (CV) for these Doppler measurements in this study were HR: 12.8%; SV: 18.7%; CO: 14.0%; PAT 2.8%; and MAP: 7.1%.

Data analysis

Comparisons were made between active and placebo treatments by repeated measures analysis of variance (ANOVA). Where the overall ANOVA was significant, Duncan's multiple range testing was used to determine differences at individual time points. A *P* value of less than 0.05 was considered significant and results are expressed as means \pm s.e.mean and where a difference between means is quoted, the 95% confidence (CI) for this difference is given.

Results

Acute administration of L-NMMA was not associated with any adverse effects during the study. Baseline conditions on each study day were similar for all haemodynamic parameters measured. L-NMMA had significant pressor effects compared with placebo, where both SVR ($P=0.02$); mean difference $479 \text{ dyn s cm}^{-5}$ (95% CI 87–872) and TPR ($P=0.02$); 63 dyn s cm^{-5} (95% CI 15–110) were elevated at t_1 and these effects persisted throughout the entire infusion period (Figure 1). Although MAP was significantly increased during infusion of L-NMMA compared with placebo at t_1 ($P=0.03$); mean difference 5 mmHg (95% CI 1–10) an effect that persisted throughout the entire infusion period, MPAP was unchanged. In addition HR ($P=0.003$); mean difference

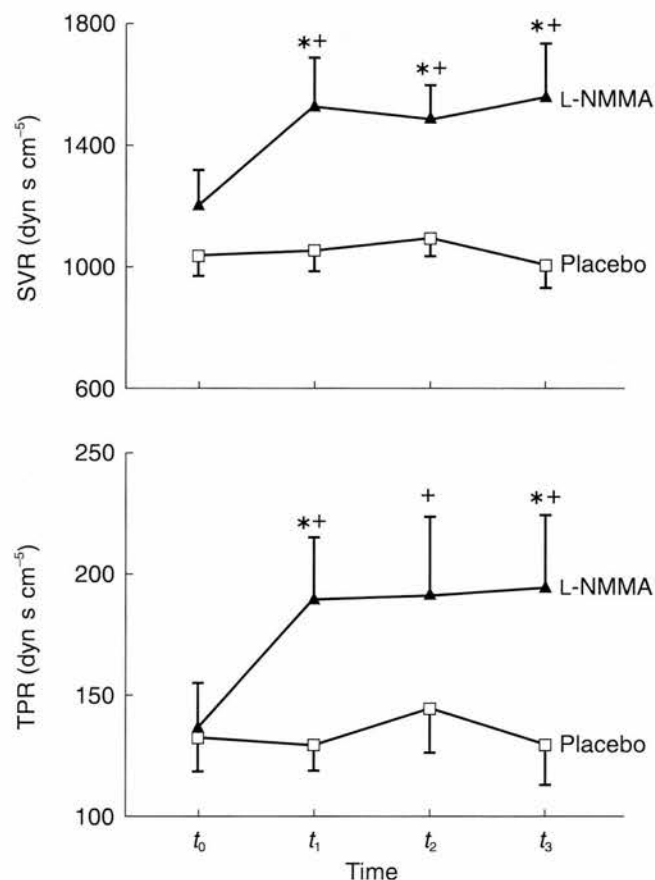


Figure 1 Systemic and pulmonary pressor effects of L-NMMA and placebo infusions in normal man. Asterisk (*) indicates significant difference between infusion with L-NMMA and placebo at that time point. Cross (+) indicates significant difference from baseline during L-NMMA infusion.

9 beats min^{-1} (95% CI 5–13) and CO ($P=0.02$); mean difference 1.74 l min^{-1} (95% CI 0.33–3.15) were significantly lower during infusion with L-NMMA compared with placebo at t_1 an effect that persisted throughout the infusion period (Table 1).

Discussion

Our results suggest that basal generation of NO is important in the regulation of both systemic and normoxic pulmonary vascular tone in normal man reflected by an increase in both SVR and TPR during inhibition of nitric oxide synthesis.

The systemic effects of NO inhibition in man are well documented. Our results however suggest that in addition that resting normoxic pulmonary vascular tone is dependent on the continuous generation of nitric oxide. In this respect infusion of L-NMMA resulted in a significant increase in TPR which was sustained throughout the entire infusion period. The lack of effect on MPAP is likely to reflect the coexistent reduction in cardiac output. Although this phenomenon has been shown in some lower mammals [7, 8] it has not been demonstrated in either rat [9] or dog models [10]. The results of this study support the findings of Stamler *et al.* [6] who demonstrated a similar effect during a dose ranging study in normal man. In addition, our results

Table 1 Haemodynamic effects of L-NMMA and placebo infusions in normal man.

		t0	t1	t2	t3
HR	P	71 ± 2	69 ± 3	70 ± 4	67 ± 3
(beats min ⁻¹)	L	68 ± 4	60 ± 4* ⁺	59 ± 3* ⁺	57 ± 3* ⁺
SV	P	102 ± 5	104 ± 6	101 ± 4	111 ± 6
(ml)	L	100 ± 6	95 ± 6 ⁺	91 ± 5 ⁺	90 ± 7* ⁺
CO	P	6.92 ± 0.47	6.91 ± 0.48	6.66 ± 0.44	7.21 ± 0.56
(l min ⁻¹)	L	6.34 ± 0.52	5.17 ± 0.5* ⁺	5.10 ± 0.41* ⁺	5.27 ± 0.52* ⁺
MAP	P	87 ± 3	89 ± 3	89 ± 3	87 ± 3
(mmHg)	L	90 ± 3	94 ± 3* ⁺	95 ± 3 ⁺	94 ± 2* ⁺
MPAP	P	11 ± 1	11 ± 1	12 ± 1	11 ± 1
(mmHg)	L	10 ± 1	12 ± 1	12 ± 2	12 ± 1

Absolute values (mean ± s.e.mean). Asterisk (*) indicates significant difference between infusion with L-NMMA (L) and placebo (P) at that time point. Cross (+) indicates significant difference from baseline during L-NMMA infusion.

suggest that these changes persist throughout a more prolonged infusion period.

We chose to use a dose of L-NMMA based on findings from previous *in vivo* studies conducted in man to ensure a good profile of nitric oxide inhibition over the whole time course of the study and found comparable systemic haemodynamic effects. Previous work by Haynes *et al.* [5] examined the maximal haemodynamic responses to increments in doses of L-NMMA. He examined on separate days doses of L-NMMA in ascending order (0.03, 0.1, 0.3, 1.0, 3.0 mg kg⁻¹) infused over 60 min and then 3 mg kg⁻¹ L-NMMA infused over 20 min then the same dose infused over 5 min via a peripheral cannula. Although, at doses > 1 mg kg⁻¹ there was an apparent decrease in heart rate, cardiac index and an increase in peripheral vascular resistance no effect on blood pressure was observed until a dose of 3 mg kg⁻¹ was given over 20 min when there were isolated increases in both systolic and diastolic blood pressure but these did not occur consistently at similar time points. He and co-workers performed the definitive study using 3 mg kg⁻¹ over 4 min and found maximal increases in mean arterial blood pressure 10 min after the start of the infusion. In a dose ranging study by Stamler *et al.* [6] the haemodynamic responses to dosages of 0.01, 0.03, 0.1, 0.3 and 1.0 mg kg⁻¹ min⁻¹ each for 3 min via a central venous catheter sheath were examined. Although an increase in mean arterial blood pressure was observed at 0.1 mg kg⁻¹ min⁻¹ infused over 3 min no effects were observed on the pulmonary vascular bed until the maximal rate of infusion 1.0 mg kg⁻¹ min⁻¹ given over 3 min. The discrepancy between effects on the systemic and pulmonary vascular beds may be explained by *in vitro* studies suggesting that L-NMMA is a relatively ineffective inhibitor of NO synthase in pulmonary blood vessels [15].

We used a front loaded infusion i.e. a combination of bolus with a continuous infusion to give a good overall profile of NO inhibition over the whole time course of the study. We infused an initial 4 mg kg⁻¹ bolus over 2 min (ie 2 mg kg⁻¹ min⁻¹ for 2 min) with a continuous infusion of 4 mg kg⁻¹ h⁻¹ to ensure that we would achieve maximal NO inhibition during the period under investigation. The maximal haemodynamic changes in this study were noted at 4 min and persisted throughout the entire infusion period. We cannot comment whether haemodynamic changes

occurred earlier than 4 min since the time scale of measurements was influenced partly by the time required for signal acquisition and measurements to be made in triplicate.

In addition for safety considerations we did not feel that a dose escalation above the infusion rate used in this study would be safe because of worries over limiting systemic hypertension and worries over precipitating coronary vascular spasm. The evidence from studies suggests that L-NMMA is a relatively ineffective inhibitor of NO synthase in pulmonary blood vessels [15] and therefore to construct a dose response curve based on the results of previous studies would have meant doubling and quadrupling our dose of L-NMMA and exposing our patients to unwanted risks.

With respect to methodology, we have used non-invasive Doppler-echocardiography to measure haemodynamic changes in the pulmonary circulation. These non-invasive techniques have been shown to be reproducible [16] and the close correlation between Doppler PAT and MPAP as measured by right heart catheter, is well established both in our own hands and those of other workers [13, 14, 17, 18]. Less, however, is known of the correlation between changes in MPAP and PAT. Using regression equations incorporating PAT, Chow *et al.* [19] examined patients before and after pulmonary thromboendarterectomy and although statistically significant the correlation between changes in PAT and catheter measures of MPAP was weak ($r = -0.41$). Beard *et al.* [20], however, showed a good correlation in 11 normal subjects made hypoxic $r = -0.73$. In addition studies performed in normal man using this non-invasive methodology concur with results of invasive studies and we therefore feel that the available evidence supports our view that PAT can be used to assess changes in the pulmonary vascular bed, particularly in normal volunteers where repeated cardiac catheterisation is viewed by some including ourselves as unethical. Doppler derived measures of cardiac output have been shown to be reproducible and correlate well with invasive measures [21]. The major source of error is generally accepted as measurement of the annular diameter and this would be mitigated by the cross-over design of this study.

There is a growing body of evidence suggesting that endothelial dysfunction is important in the development of hypoxic pulmonary hypertension. Endothelium dependent

relaxation is reduced in chronically hypoxic rats [11] and in man in end stage chronic hypoxic lung disease endothelium dependent pulmonary artery relaxation is impaired and in addition the contractile responses to pressor stimuli increased [12]. These patients are known to have elevated levels of pressor substances such as endothelin-1 [22], which has been shown to be potent pressor agent in man *in vivo* [23], and may have significantly greater pressor effects in the context of a dysfunctional endothelium.

In addition to pressor effects we also noted a reduction in HR, CO and SV. This may have been mediated via a baroreceptor reflex and increase in left ventricular afterload although a role for nitric oxide generation in the maintenance of cardiac output remains a possibility.

To conclude, we have shown that basal nitric oxide generation is important in maintaining basal vascular tone. In disease states an imbalance of constricting and relaxing factors may contribute to both systemic and pulmonary vasoconstriction. The development of therapies which are able to deliver relaxing factors such as nitric oxide to the site of endothelial dysfunction holds promise, particularly in chronic hypoxic lung disease where effective therapies are limited.

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